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(FIZIOLOGIIA RASTENII)

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TRANSLOCATION OF SUGARS IN GRAFTED PLANTS

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Investigations on the translocation of organic materials in plants have been more intensive in the past year. The growing interest in this problem is due to the importance of the translocation processes themselves in the life of the plant as well as to the possibilities for direct observation of translocation with radioactive materials [1] and fluorescent dyes [2] which have arisen. Moreover, with the attention of plant physiologists turning more and more during this period to the problem of the integrity of the plant organism [3], the lack of knowledge of the mechanism and pattern of translocation, i.e., those processes by means of which metabolic products are exchanged between the individual plant organs, has been keenly felt.

At the present time the majority of investigators are agreed that translocation in the phloem is in some way connected to the activities of living cells; the nature of this relationship as well as the mechanism of translocation itself is still debatable.

A more accurate description of the nature of the translocated compounds and also the study of those transformations to which such materials may be subjected in the conducting tissues might have considerable significance for the understanding of the mechanism of translocation.

Mason and Phillis [4], on the basis of an analysis of the cortex of cotton plants, have assumed that the chief transport form of sugar in this plant is sucrose.

The validity of this assumption was demonstrated in a study of Kursanov and Vyskrebentseva [5] with C^{14}-labeled assimilates in which it was observed that despite the complete absence of sucrose in the growing fibers of cotton this sugar was predominant in the conducting elements serving these fibers. According to the data of Vernon and Aronoff [6], sucrose is the chief compound transported in the phloem of soybean, and according to the observations of Pavlinova [7] and Turkina [8] the same sugar predominates in the fibrovascular bundles of sugar beet. Thus, in the light of these data, sucrose is the chief transport compound in many plants.

Radioautographs show that assimilates moving from the mesophyll to the conducting tissues go directly to the site of utilization or deposition of reserves and, at least in the first hours, are not distributed to the parenchymatous tissues surrounding the conducting system. Therefore the radioactive compounds observed in the cortex or in sections of the leaf petioles and stems during short term experiments may usually, with one reservation, be associated with the mobile components, in contrast to the total mass of nonradioactive materials comprising the more stable reservoir of plastic materials characteristic of all tissues.

The reservation consists mainly in the fact that the fibrovascular bundles and even the phloem itself are not yet homogeneous with respect to cellular composition. The developing tissues have different physiological functions although they interact to some extent. However, since the amount and kind of participation of the individual cells of the phloem in translocation through the sieve tubes is still unclear, we are justified in regarding the phloem or the fibrovascular bundles as a whole as natural systems specialized for translocation.

Nevertheless, in the study of the composition of radioactive materials in the conducting tissues, we must keep in mind that even in this highly specialized system not all radioactive compounds are being translocated, since there occur in the sieve tubes, in addition to translocation, metabolic transformations which are characteristic

not only of the sieve tubes but also of the other cells in the conducting system which interact with them. This particular kind of phenomenon was observed by Pavlinova [7] in the fibrovascular bundles of sugar beet, in which C^{14}-fructose was converted to glucose and sucrose.

The possibility is not excluded that in plants with different types of metabolism secondary transformations of the mobile materials are specific to a given species, i.e., they will be different. However, if the transport of a molecule be regarded as a specialized metabolic process of conductive tissues, and therefore as general as glycolysis or the tricarboxylic acid cycle, it may be expected that the materials involved in this process are limited to one or only a few compounds common to many and perhaps to all species of plants.

These considerations led us to undertake an investigation on the translocation of carbohydrates in grafted plants, the components of which differ sharply in the character of their carbohydrate metabolism. Such components preserve their basic metabolic patterns, but in the event of a good graft union, act as a unit in the transport of water, mineral elements and organic compounds. Therefore, by comparing the composition of ratioactive carbohydrates in conducting tissues of the different graft components, we hoped to identify elements common to all components which are more closely involved in translocation, and elements characteristic of the individual components which are involved in secondary transformations.

METHODS

Plants of sunflower (variety Saratov 169) and Jerusalem artichoke (variety Red Kiev), which are basically different in their carbohydrate composition, were used for graft experiments (see Table 1 below).

Seeds of sunflower were planted May 30, and Jerusalem artichoke tubers were planted June 1, 1955, in earthen pots of soil in a growing house; on July 13 grafts of sunflower on Jerusalem artichoke and Jerusalem artichoke on sunflower were made; the stock was decapitated and the wedge-shaped base of the scion inserted in a notch on the cut surface; insulated wire was wound around the stem at the site of the graft. Grafted plants were kept for 5 days in a humid section of the greenhouse until the graft union was complete. Grafts were again made on August 10 in which Jerusalem artichoke was grafted to previously grafted plants with a sunflower scion, and sunflower was grafted to plants with a Jerusalem artichoke scion. Plants of the following composition were thus obtained: Jerusalem artichoke – sunflower – Jerusalem artichoke (J-S-J) (Fig. 1) and sunflower-Jerusalem artichoke-sunflower (S-J-S) (Fig. 2). The length of the sunflower component of the first grafted plant was 20-25 cm; that of the Jerusalem artichoke component of the second grafted plant was 15-20 cm.

We used such double-grafted plants for a study of the behavior of assimilates traveling downward from the leaves of the upper component through the conducting tissue of the intercalated component to the root of the original species.

The first samples for determination of carbohydrate composition were taken from control plants of both species on August 11. Samples from the grafted plants (J-S-J) and (S-J-S) were taken on September 12, 13, 14, 15 and 16, 1955.

Usually in the hour before an experiment with grafted plants all leaves except those of the uppermost component were removed. Following this one of the upper leaves was exposed for 20 minutes to radioactive carbon dioxide in a chamber at a concentration of 3% with an activity of 25 μc. Plants were then placed in the dark. Grafted plants (J-S-J) were kept in the dark 4 hours, while plants (S-J-S), in which translocation is sluggish, were kept in the dark an additional 14 hours in certain experiments. At the end of this time the stem was cut into 10–12-cm pieces. These were: 1) an upper section taken just below the leaf exposed to $C^{14}O_2$; 2) a section taken from the intercalated stem section; 3) a section from the stem of the basal stock. The cortex was rapidly stripped from these sections and the amount of various carbohydrates as well as their radioactivity determined. Moreover, in certain experiments we analyzed the basal portion of the stem and the roots (in sunflower) or the tubers (in Jerusalem artichoke) in order to obtain a more complete picture of the downward movement of assimilates.

The material was fixed in 96% boiling ethyl alcohol and then the ground tissue was extracted twice with 80% ethanol. The plant residue was extracted with water three times at 45°. Water soluble carbohydrates (glucofructosans) in the pooled water extracts were determined after 30 minutes' hydrolysis with 2% HCl at 70°. Sugar determinations were carried out using Bertrand's method.

The alcoholic extracts containing monoses, sucrose and oligosaccharides were pooled, evaporated under a vacuum, treated with $Pb(CH_3COO)_2$ and Na_2SO_4 and taken up in 3-4 ml of water. The purified solutions were used for quantitative chromatographic separation of sugars on paper [7, 9]. After elution from the paper the content of sugars and oligosaccharides was determined with anthrone.

For analysis of radioactive samples a double purification of the vacuum-evaporated solutions with ion exchange resins was carried out. This was done in order to free the extracts of radioactive amino acids, many of which have the same R_f as sugars and may thus give an erroneously high value for radioactivity of sugars. For this purpose a cation exchange resin KU-2 was used. A special run showed that this resin retains lysine, histidine, cystine, asparagine, serine, glycine, aspartic acid, glutamic acid, threonine, alanine and 90% arginine, i.e.,

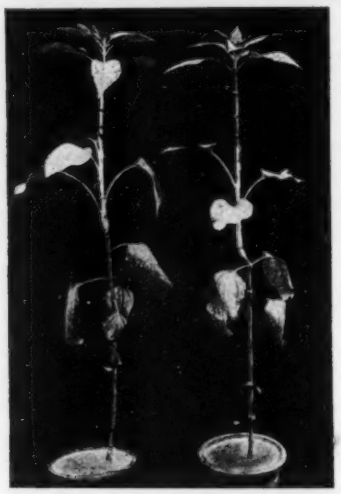

Fig. 1. Grafted plants of type Jerusalem artichoke – sunflower – Jerusalem artichoke (J-S-J) with leaves on all graft components (Photographed September 12, 1955).

Fig. 2. Grafted plants of type sunflower – Jerusalem artichoke – sunflower (S-J-S) with leaves on the middle and upper components. Sunflower scion is flowering. (Photographed September 12, 1955).

those amino acids which move with sugars in the developing solution used – n-butanol – acetic acid – water (4:1:5). Since the separation of sugars and oligosaccharides on paper takes a long time (up to four days), radioactive organic acids run off the chromatograms and do not interfere with the determination of sugar radioactivity.

For such determinations soluble sugars and oligosaccharides were eluted from the chromatogram with water (without developing spots); the eluates were concentrated, applied to disks, and counted with an end counter. Ordinarily an amount of eluate containing 2.0-3.0 mg of sugar was placed on the 3 cm^2 disks. If the eluate contained a smaller quantity of sugar, then nonradioactive sugar was added prior to transfer to the disks to raise the sugar concentration to the desired level. With the sample thickness used which was not greater than 1 mg/cm^2, a self absorption effect was usually not observed.

The data on radioactivity presented in the tables are obtained by a direct count in an end counter; the efficiency of this count was 9%. Therefore, the true values for radioactivity were about 10 times those given.

RESULTS

As was previously stated, the purpose of this investigation was to follow in grafted plants, the components of which differ with respect to carbohydrate metabolism, the change in radioactive carbohydrates during their movement from the conducting system of one component into the conducting system of the other. Using for this purpose a double-grafted plant, either (J-S-J) or (S-J-S), we were also able to estimate the degree of reversibility of these changes.

However, it was first necessary to establish the existence of the characteristic differences in carbohydrate composition of the cortex of sunflower and Jerusalem artichoke, and to then determine whether these differences are maintained in the grafted plants.

TABLE 1

Content of Sugars and Oligosaccharides in the Cortex of 70-Day-Old Sunflower and Jerusalem Artichoke Plants
Cortex Samples Taken from the Middle Portion of the Stem; Extraction with Hot 80% Ethanol (in mg per 1 g dry weight)

Plants	Glucose	Fructose	Sucrose	Oligosaccharides		
				1	2	Slowly moving compounds
Sunflower	9.10	5.90	5.50	1.10	0.90	1.70
Jerusalem artichoke	0.34	0.10	5.94	2.55	2.85	29.50

Fig. 3. Chromatograms of sugars and oligosaccharides of the cortex of Jerusalem artichoke (a) and sunflower (b); f) fructose, s) sucrose, 1-8) oligosaccharides in order of increasing polymerization.

The results of a comparative analysis of sugars and oligosaccharides in the cortex of 70-day-old plants of sunflower and Jerusalem artichoke are shown in Table 1.

Chromatographic separation on paper revealed the presence in the cortex of both plants of glucose, fructose, and sucrose, and in addition a group of oligosaccharides which are divided into three groups: first, the most simple oligosaccharide which is closely related to surcose; second, another mobile oligosaccharide; third, a group of more highly polymerized compounds which do not migrate far from the origin with the solvent system used, n-butanol – acetic acid – water (4:1:5).

A comparison of these data shows that while the sucrose content is approximately equal in the two plants, monoses (glucose and fructose) predominate in the sunflower and oligosaccharides in Jerusalem artichoke.

These and other compounds may be regarded as products directly related by their origin through transformations of sucrose, which may be hydrolytically cleaved to monosaccharides by invertase action or which may yield first oligosaccharides and then more complex glucofructosans as a result of the transferring action of invertase [10-13] or inulosaccharase [14, 15]. The characteristic distinction between sunflower and Jerusalem artichoke consists in the fact that in the cortical tissues of the former, products of the hydrolytic cleavage of sucrose are chiefly accumulated, and in the latter – products of transfructosidation. We may graphically illustrate these differences in the following manner:

This difference in carbohydrate composition is also clearly evident on chromatograms of sugars and oligosaccharides from the cortical tissues of the two species (Fig. 3). Further experiments indicated, moreover, that this difference is maintained in grafted plants even though a common conducting system through which plastic materials move has developed.

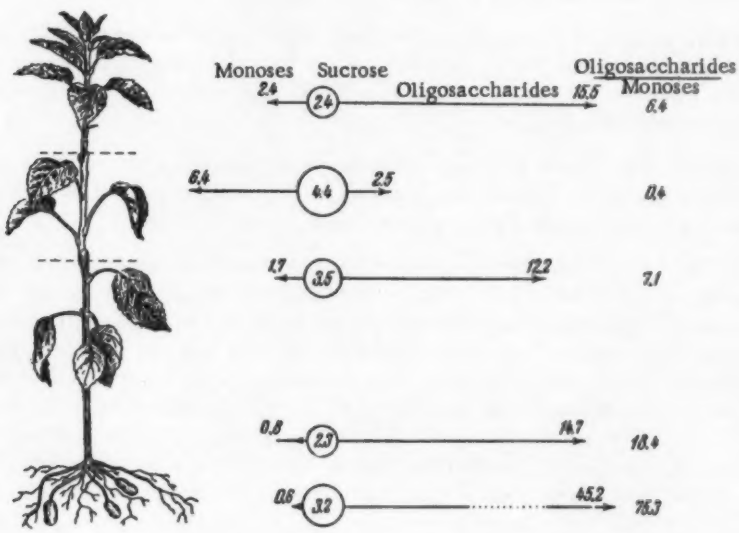

Fig. 4. Relative content of monosaccharides and oligosaccharides in the cortex and tubers of the grafted plant Jerusalem artichoke – sunflower – Jerusalem artichoke.

The results of one such experiment carried out with the double-grafted plant (J-S-J) are presented in Table 2 and Fig. 4.

TABLE 2

Carbohydrate Content in the Cortex and Tubers of the Double-Grafted Plant Jerusalem Artichoke – Sunflower – Jerusalem Artichoke (in mg per 1 g dry weight; J) Jerusalem Artichoke; S) Sunflower)

Plant species (from top to bottom)	Material analyzed	Glucose	Fructose	Sucrose	Oligosaccharides					Water soluble gluco-fructosans
					1	2	3	4	5-8	
J	Cortex above intercalated stem	1.6	0.8	2.4	0.7	1.2	1.6	2.0	9.0	12.6
S	Cortex of intercalated stem	4.1	2.3	4.4	0.7	0.5	0.4	0.3	0.6	0.7
J	Cortex below intercalated stem	1.0	0.7	3.5	1.2	1.5	1.4	1.9	6.2	27.4
J	Cortex of stem base	0.2	0.6	2.3	0.9	1.5	2.0	1.8	8.5	34.8
J	Tubers	0.3	0.3	3.2	3.8	4.8	6.6	4.0	26.0	81.5

The table shows that in the cortex of Jerusalem artichoke, from the apex of the stem to the tubers, there is a quite regular pattern of change in carbohydrate composition which is expressed in a decrease in monose content (mainly glucose) and in the accumulation of oligosaccharides (predominantly the more highly polymerized). The sucrose level does not change in any regular fashion along the plant axis. These results are as a whole in agreement with the data of Strepkov [16] who confirmed in ungrafted Jerusalem artichoke plants an increase in the content of glucofructosans from the stem apex to its base.

The data of Table 2 also show that the succession of changes in carbohydrate composition characteristic of Jerusalem artichoke is abruptly interrupted for almost all sugars in the intercalated sunflower stem. Here there is an accumulation of monoses and a decrease of almost all oligosaccharides, i.e., the predominance of monosaccharides which is typical of sunflower is apparent (see Table 1).

The maintenance of the characteristic carbohydrate equilibria in each of the graft components may be more vividly shown graphically (see Fig. 4). On the diagram the sucrose content (in mg) is expressed by the diameter of the circles, while the total amount of monoses and of oligosaccharides is designated by the length of the corresponding horizontal lines.

It is evident from this diagram (see Fig. 4) that the ratio oligosaccharides/monosaccharides, which for the cortex of Jerusalem artichoke has a manifest tendency to increase in the direction of the tubers, is sharply reduced in the cortex of the intercalated sunflower stem.

In the experiment under discussion the mixture of oligosaccharides soluble in 80% ethanol was chromatographically separated into four individual substances (1-4) and a group of four poorly resolved compounds. These compounds migrated to positions between sucrose and the origin in accordance with molecular size, the most simple (1) oligosaccharide moving just behind sucrose and the most complex (5-8) remaining close to the origin. In addition, the still more highly polymerized glucofructosans, which are insoluble in alcohol, were extracted with water and determined separately (see Table 2). Usually the formation of oligosaccharides under the influence of invertase [13] and especially inulosaccharase [14] proceeds in the direction of increasing complexity from a molecule consisting of glucose and two fructose residues to compounds in which the fructose residues form a chain of variable length. One of these reactions may be represented thus:

1. Sucrose + sucrose → (Glucose – fructose – fructose) + glucose

2. (Glucose – fructose – fructose) + sucrose → (glucose – fructose – fructose – fructose) + glucose, etc.

The fructose-transferring activity of invertase is small in proportion to its hydrolytic activity and is, moreover, limited to the formation of 2-4 of the most simple oligosaccharides [10, 13]. More highly polymerized oligosaccharides in greater quantities are formed, apparently, under the influence of inulosaccharase [14]. It is,

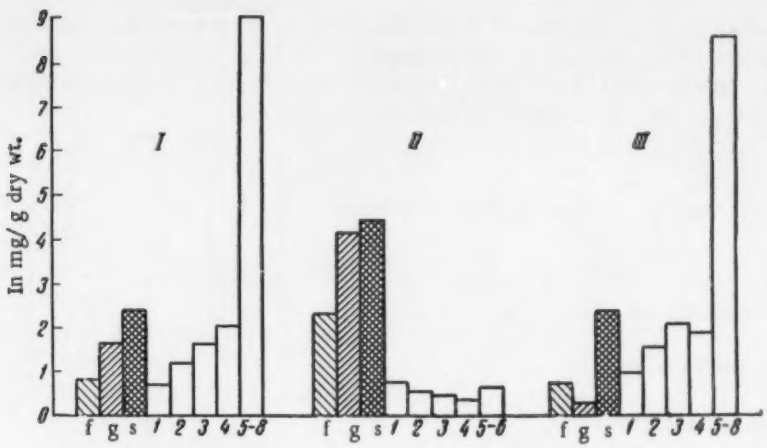

Fig. 5. Oligosaccharide composition of the cortex of the grafted plant (J-S-J). I) Jerusalem artichoke (upper component); II) sunflower (intercalated component); III) Jerusalem artichoke (stock); f) fructose, g) glucose, s) sucrose; 1, 2, 3, 4, 5-8) oligosaccharides in order of increasing polymerization.

thus, extremely probable that more than one enzyme participates in the formation of oligosaccharides.

In connection with this it is interesting to note that the cortical tissues of Jerusalem artichoke and sunflower differ not only in the amount of oligosaccharides present, but also in the composition of this fraction. Actually, while in the Jerusalem artichoke highly polymerized compounds are being formed by transfructosidation reactions going through an increasingly complex series of oligosaccharides, in the sunflower this process is limited primarily to the formation of simple oligosaccharides. This is illustrated by the data presented in Fig. 5.

The synthesis of oligosaccharides in a simple system should be accompanied by the disappearance of sucrose and the accumulation of glucose which is released in the transfer of the fructose residue. However, this relationship is weakly expressed in the whole plant, probably because of sugar translocation and isomerization of monoses (see Table 2).

The material presented above leads to the basic conclusion that in the grafted plant (J-S-J) the carbohydrate composition of the cortex has the same characteristic features as that of the plant species from which the graft component came, in spite of the fact that the phloem of such a plant forms a single conducting entity.

This result does not, however, lead to the conclusion that the translocated carbohydrates are different in the two plants and that such compounds entering the conducting system of one graft component from that of the other undergo an immediate radical change. It is undoubtedly true that in such a complex tissue system as that of the cortex a total carbohydrate analysis would not reveal the nature of the compounds contained in the conducting elements of the phloem, and certainly not the nature of the substances being translocated.

A study of the radioactivity of individual sugars might bring us much closer to a solution of this problem, since radioactive carbohydrates moving through the cortex from the leaf exposed to $C^{14}O_2$, at least in short term experiments, should be mainly concentrated in the conducting cells and should be in part involved actively in translocation.

The determinations of radioactivity of individual carbohydrates in the cortex of the grafted plant (J-S-J) which we carried out with this purpose are presented in Table 3.

As the data of Table 3 show, the greatest radioactivity is concentrated in sucrose throughout the stem length, although the total sucrose content is relatively low in both plants (see Table 2).

For example, about 80% of the carbohydrate radioactivity is found in sucrose in the cortex of the upper Jerusalem artichoke component, although this compound comprises less than 8% of the total carbohydrates by

TABLE 3

Radioactivity of Carbohydrates in the Cortex of the Grafted Plant Jerusalem Artichoke – Sunflower – Jerusalem Artichoke After Exposure of the Leaves of the Upper Component to $C^{14}O_2$
(Exposure to 3% CO_2 enriched with $C^{14}O_2$ (25 μc) – 20 min. in natural daylight, time of translocation – 4 hours in darkness; in counts/min per 1 g dry weight of the cortex)

Plant species (from top to bottom)	Material analyzed	Glucose	Fructose	Sucrose	Oligosaccharides					Water soluble gluco-fructosans
					1	2	3	4	5-8	
J	Cortex above intercalated stem	1075	754	15130	1430	155	30	30	60	247
S	Cortex of intercalated stem	213	195	5730	102	15	9	0	0	155
J	Cortex below intercalated stem	64	68	2390	180	8	0	0	0	36
J	Cortex of stem base	11	7	642	31	0	0	0	0	8
J	Tubers	0	0	27	0	0	0	0	0	0

TABLE 4

Ratio of Radioactivities of the Principal Groups of Carbohydrates in Various Parts of the Cortex of the Grafted Plant Jerusalem Artichoke – Sunflower – Jerusalem Artichoke

Plant species (from top to bottom)	Material analyzed	Ratio of radioactivities	
		Sucrose: total monoses	Sucrose: first oligo-saccharide
J	Cortex above intercalated stem	8.3	11.0
S	Cortex of intercalated stem	14.0	56.0
J	Cortex below intercalated stem	18.0	13.0
J	Cortex of stem base	36.0	20.0

weight. We found approximately as significant a concentration of radioactivity in sucrose in other sections of the stem (up to 90%).

The data of Table 3 also show that the ratioactivity of all carbohydrates rapidly declines as the distance from the treated leaf increases. This indicates, presumably, that labeled assimilates are still being translocated 4 hours after exposure of the leaf to $C^{14}O_2$, and that they have just begun to appear in the tubers where a small amount of labeled sucrose is already present. The lack of completion of translocation of assimilates in experiments of this kind has a great importance, since this guarantees that a portion of the radioactive materials are still being translocated.

Since the outflow of assimilates in this plant was quite sluggish, it is probable that in the upper cortical tissues, where assimilates appeared earlier, secondary transformations might have proceeded to a greater extent than in tissues further down the stem.

In Table 4, based on data from the same experiment, are presented ratios of the radioactivity of sucrose, which we designate as the chief transport material, to the radioactivity of the mono- and oligosaccharides accompanying it. In these calculations, for the monose fraction the total radioactivity of glucose and fructose were taken because of the rapid isomerization of monoses in the plant, and for the oligosaccharide fraction only the first oligosaccharide, which is most closely related to sucrose and as a consequence the most radioactive, was taken.

As the data of Table 4 show, the ratio of radioactivities sucrose/total monoses increases from the exposed leaf to the stem base. This indicates a relative increase of labeled sucrose as the assimilates move downward through the conducting system. This may be a result of a partial conversion of monoses to sucrose, as was shown by Pavlinova [7] for the conducting tissues of sugar beet. On the other hand, the increase in this ratio may

be brought about by a more rapid translocation of sucrose [6, 17], which would require, however, the assumption of independent movement of each of the carbohydrate components.

It is also extremely important that the regular increase in this ratio is not disturbed during assimilate flow through sunflower phloem although, judging from the total carbohydrate composition of this graft component (see Table 2), a decrease due to an equilibrium shift toward monoses might be expected. All this leads to the conclusion that the ratio of sucrose to monosaccharides in the conducting system depends to only a slight extent on the plant species and the nature of the carbohydrate complement of the surrounding tissues and, consequently, that the changes observed occur in the conducting system itself, which functions in the grafted plant as a single unit.

As for the radioactivity ratio sucrose/first oligosaccharide, it steadily increases downward in the conducting system of Jerusalem artichoke, indicating a relative increase of labeled sucrose as assimilates move from the stem apex to the base. In this case, however, as Table 4 shows, during passage of the carbohydrates through the sunflower graft component, the ratio increases sharply (five times) as a result of decreased level of oligosaccharides, and then increases again upon entrance of assimilates into the lower Jerusalem artichoke graft component. It is thus evident that the species of the graft component involved is of basic importance with respect to the radioactivity ratio sucrose/first oligosaccharide in the conducting system.

Proceeding from the earlier conclusion that the composition of sugars in the conducting tissues depends to only a slight extent on the metabolic character of the surrounding tissues, we may propose for this case that the formation of oligosaccharides takes place not in the conducting cells themselves but in the accompanying tissues into which the translocated sugars move to some degree. It is further possible that the oligosaccharides themselves do not in general undergo translocation, but are simply stored in greater or less quantity in the surrounding tissues according to the nature of the plant.

The second series of experiments was carried out with grafted plants in which the component parts were reversed, i.e., the plant (S-J-S) was used. Graft unions of such plants do not form as readily as those of plants of the first type. As the experiments with $C^{14}O_2$ showed, the flow of assimilates through the graft unions was very sluggish. Nevertheless, a body of precise data substantiating the basic conclusions arrived at in the experiments with (J-S-J) plants was obtained with these plants.

So, for example, the carbohydrate composition of a graft component characteristic for its species was maintained to the same extent in plants of the second type as in those of the first type. This is expressed by a relative predominance of monoses in the sunflower cortex, and of oligosaccharides in the Jerusalem artichoke cortex. It is schematically shown in the diagram of Fig. 6, which is based on one such experiment.

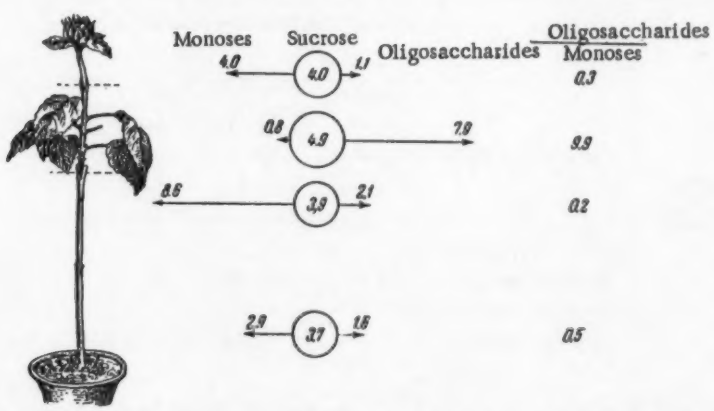

Fig. 6. Relative content of mono- and oligosaccharides in the cortex of the grafted plant sunflower – Jerusalem artichoke – sunflower.

The character of the oligosaccharide complement in such plants is quite similar to that of J-S-J plants, with the Jerusalem artichoke cortex containing relatively more polymerized compounds (Fig. 7).

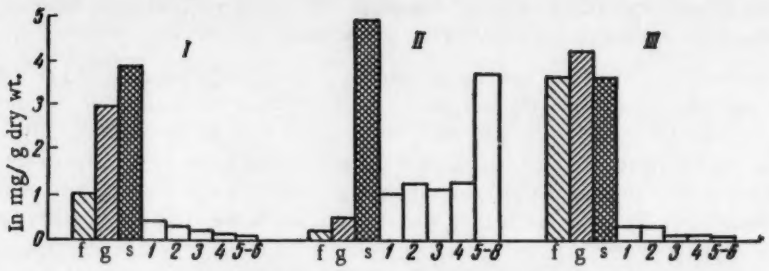

Fig. 7. Composition of oligosaccharides in the cortex of the grafted plant (S-J-S). I) Sunflower (upper component); II) Jerusalem artichoke (intercalated component); III) sunflower (stock); f) fructose, g) glucose, s) sucrose; 1, 2, 3, 4, 5-8) oligosaccharides in order of increasing polymerization.

TABLE 5

Radioactivity of Carbohydrates in the Cortex of the Grafted Plant Sunflower – Jerusalem Artichoke – Sunflower (S-J-S) After Exposure of the Leaves of the Upper Component to $C^{14}O_2$
(Exposure to 3% CO_2 enriched with $C^{14}O_2$ (25 μc) – 20 min; outflow 14 hours in the dark, in counts/min/g dry weight of the cortex)

Plant species (from top to bottom)	Material analyzed	Glucose	Fructose	Sucrose	Oligosaccharides	
					First	Others
S	Cortex above intercalated stem	756	538	8970	185	80
J	Cortex of intercalated stem	73	43	1143	81	7
S	Cortex below intercalated stem	0	20	37	0	0

TABLE 6

Radioactivity Ratios of Principal Groups of Carbohydrates From Various Parts of the Cortex of the Grafted Plant (Sunflower – Jerusalem Artichoke – Sunflower)

Plant species (from top to bottom)	Material analyzed	Sucrose: total monoses	Sucrose	
			First oligosaccharide	
			Experiment 1	Experiment 2
S	Cortex above intercalated stem	6.8	48	30
J	Cortex of intercalated stem	9.5	14	7

In view of the sluggish flow of assimilates in this plant, we were unable to follow the movement of radioactive materials to the stem base although in certain experiments we kept the plants 14 hours in darkness after exposure to $C^{14}O_2$.

Data from one of the experiments on S-J-S plants are presented in Table 5.

These data show that here the overwhelming part of the radioactivity is concentrated in sucrose (approximately 85% of total carbohydrate radioactivity) and that this is independent of the nature of the graft component involved, even though a sunflower leaf assimilated the $C^{14}O_2$ and not a Jerusalem artichoke leaf as in the previous experiments. This is in good agreement with results of other authors [18-20], who showed with $C^{14}O_2$ that in widely different plants the first free sugar formed in photosynthesis is sucrose. Thus, appearing in convincing measure to be the universal product of photosynthesis and, moreover, being evidently capable of easy movement from the mesophyll to the phloem cells, sucrose shows itself to occur universally in the conducting system of the various plants [5, 7, 21, 22].

Finally, comparing the ratios of sucrose radioactivity to the radioactivity of accompanying sugars in the conducting system of various parts of the cortex, we came to the conclusion that in this plant the mixture (sucrose – monoses) becomes relatively richer in sucrose as one proceeds downward along the stem (as a result of its synthesis from monoses or a more rapid translocation) (Table 6).

At the same time, transport of this mixture from one graft component to another (in this case from sunflower to Jerusalem artichoke) is not associated with any marked changes in the radioactivity ratio of sucrose to monoses, which confirms the view that these sugars in the conducting system are for the most part isolated from the influence of surrounding tissues.

The radioactivity ratio sucrose/first oligosaccharide exhibits a strong dependence on the nature of the plant in which the labeled assimilates occur. As in the first experiment (see Table 4), this ratio was very high in sunflower cortex and then abruptly dropped several times with transport into the Jerusalem artichoke cortex.

Thus, the tissues of the different graft components exert a marked influence on the distrubition of radioactivity between labeled sucrose and oligosaccharides. However, as has already been noted, it is possible that these transformations are not immediately connected with the vital activities of the conducting cells, but take place in the surrounding tissues into which a part of the moving sucrose enters.

Judging from all these facts, the composition of the sugar complement of conducting tissues depends to a comparatively slight extent on the nature of the assimilating leaf or the metabolic properties of the surrounding tissues. Evidently, the specific sugar transformations characteristic of one or another plant species take place subsequent to migration of sugars out of the conducting cells. This conclusion is concerned, however only with sugars, and does not exclude the possibility that exchange of more specific products between the graft components may occur.

SUMMARY

To investigate sugar translocation we used double grafted Jerusalem artichoke sunflower Jerusalem artichoke (J-S-J) and sunflower Jerusalem artichoke sunflower (S-J-S) plants in which a common conducting system of plastic substances is formed by the fusion of phloem of the different graft components.

It is shown that the grafted parts of plants retain the carbohydrate content characteristic of the given species. This is seen in the relative predominance of monosaccharides in the cortex of the sunflower and in the predominance of oligosaccharides in the cortex of Jerusalem artichoke (Tables 1, 2 and Fig. 4, 6). Besides, Jerusalem artichoke differs from sunflower by a relatively higher content of high polymer glucofructosans. Fig. 5 and 7). Sucrose is characteristic of both species to about the same extent.

$C^{14}O_2$ supplementary nutrition of the leaves of the upper component shows that sucrose is the predominant radioactive sugar in the conducting system of the cortex along the whole length of the stem. From 80 to 85% of total carbohydrate radioactivity is in sucrose.

Sucrose is the predominant sugar in the conducting system of either of the grafted components irrespective of whether the labelled assimilates were formed in the leaves of Jerusalem artichoke or in the leaves of sunflower. (Tables 3 and 5).

As the assimilates move through the conducting tissues, the radioactivity ratio sucrose/monosaccharides increases. However, the ratio of sucrose to monosaccharide does not change radically when the assimilates pass from one species into another. This indicates a certain lack of dependence of the composition of sugar in the conducting cells on the metabolic peculiarities of the surrounding tissues. (Tables 4 and 6).

The ratio of sucrose to oligosaccharide radioactivity on the contrary, shows a marked variation with the tissue metabolism. This change corresponds to the specific carbohydrate composition of the given plant. Therefore it is assumed that synthesis of oligosaccharides does not take place in the conducting cells themselves but in the surrounding tissues into which sucrose may partially penetrate while moving along the conducting cells.

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PRESOWING FUMIGATION OF CUCUMBER SEEDS WITH CARBON MONOXIDE

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Among the crop plants cultivated by man there are many monoecious and dioecious ones. Treatment procedures which alter the ratio of the sexes and increase the development of pistillate flowers are very important in increasing the yield of these plants. The problem of sex reversal in these plants has recently been extensively expanded.

The work of several investigators [1-4] has shown that reversal in the sex characteristics of a plant because of the influence of external environmental factors is due basically to a change in the metabolism and the direction of the physiological processes. The work by Minina [4] showed that due to such external factors as temperature, soil and air moisture, mineral fertilizer, and the effect of gases, the water relations of a plant, the oxidation–reduction processes in the cells, respiration, and other physiological processes change. These changes exert a great influence on the formation of sexual characteristics in a plant.

Some investigators have succeeded in obtaining positive results in attempting to change the sexual characteristics of plants by using carbon monoxide, carbon dioxide, and other gases. All the investigations concerning the effect of gases on the sexual characteristics of plants revealed that gases produce a change in the sex of the plant, and most frequently this is in the pistillate direction [3-6]. The procedure of treating plants with various gases has been named plant fumigation.

The fumigation of cucumbers with carbon monoxide has long been used during greenhouse cultivation of this crop. This method was worked out by trellis gardeners and received the name of cucumber "smoking". A description of the trellis method for raising cucumbers can be found in several of the guides to raising vegetables [7, 8].

Cucumbers are "smoked", i.e., kept in an atmosphere enriched with carbon monoxide, in order to obtain more pistillate flowers. Trellis gardeners "smoke" the seedlings or subject the plants to "smoking" in the vegetative phase, before flowering. Edel'shtein [8] showed that after such treatment there were sometimes practically no staminate flowers.

Minina's [4] work concerning the fumigation of cucumbers during the vegetative phase of development revealed that this practice brought about a significant shift of the sex toward the pistillate direction, however fumigating with carbon monoxide gas inhibited the growth processes.

Instead of fumigating the cucumbers during the vegetative phase, we fumigated germinated seeds and decided to check this method on plants grown in field conditions. Young plants, according to I. V. Michurin, respond more readily to changes caused by external environmental factors. It appears that the effect of carbon monoxide on the plants at a very early period of growth during the vernalization stage should affect the metabolism of the fumigated plants, produce a change in their biology, and change their sexual characteristics.

The positive data obtained in our experiments should be useful in introducing a method of presowing fumigation of cucumber seeds during their cultivation in the greenhouse, as well as in the field.

METHODS

Cucumber seeds were soaked in water at room temperature (18-20°) for twenty-four hours, following this

they were kept in a desiccator saturated with carbon monoxide gas for another twenty-four hours.

There is some indication that vernalization of cucumbers occurs within three to four days at a temperature of 18-20° [9]. Consequently, during fumigation of the cucumber seeds vernalization occurred, if not completely at least partially, in an atmosphere enriched with carbon monoxide. The controls consisted of plants obtained from seeds which were soaked together with the experimental ones, but which developed later at normal conditions. After the treatment the control and experimental seeds were planted in duplicate in field plots having an area of 3 m^2.

Carbon monoxide was obtained from the dissociation of formic acid. We tried to duplicate the fumigation method used in "smoking" the cucumbers which is used in greenhouses, hence we didn't deem it necessary to give the plants a specific percentage of gas. After computing the volume of the desiccator, we calculated the amount of formic acid required in order to obtain the amount of gas necessary to completely saturate the desiccator. Taking into account the fact that during such a fumigation procedure there will be an inevitable loss of gas, we considered that this amount would be adequate to bring about a reaction on the seeds. According to Minina [4] 1% of the gas in the air is sufficient for a reaction.

Two varieties of cucumbers were used as experimental material: the variety Muromskie, an early maturing one in the environs of the Yaroslav region, and the variety Nerosimye, a late maturing one. Both varieties were fumigated at the same time and for the same length of time.

The experiments were carried out in the Kotorostli river valley within the boundaries of the city of Yaroslav. During that year cucumber seeds variety Nerosimye were planted at the "Voskhod" collective farm after they had received presowing treatment with carbon monoxide gas, and in 1955 cucumber seeds variety Viaznikovskie which had also been treated with carbon monoxide were planted in hotbeds at the Botanical Garden of the Pedagogical Institute.

Phenological observations were made on the experimental and control plants at field conditions, the number of flowers was determined by systematically removing the expanded flowers after two days, and the various physiological processes were studied.

A study of the photosynthetic process using the twin-leaf method accompanied by the calculation of the flow of materials from the leaves revealed that presowing fumigation of cucumber seeds with carbon monoxide had affected the photosynthetic process during the ontogeny of the plant (Table 1).

Fumigation induced a change in the rate of photosynthesis in both varieties; during the beginning of growth the rate of photosynthesis was lower in the fumigated plants than in the controls, but near the period of fruit production a change occurred and photosynthesis proceeded more vigorously in the fumigated plants. There was a more vigorous accumulation of materials in the Nerosimye variety but in the Muromskie variety there was a greater flow of materials from the leaves.

The respiration process also occurred differently in the fumigated plants. Respiration was measured in hermetically sealed glass chambers according to Boysen-Jensen's method (Table 2).

It is evident from the data in Table 2 that in the Nerosimye variety the rate of respiration was slower in the fumigated plants than in the controls during the entire season. No decrease in respiration rate was observed in the Muromskie variety.

Presowing fumigation of the seeds with carbon monoxide induced a change in the colloidal properties of the protoplasm of the cucumber plants by increasing the hydrophilic properties of the colloids; this expressed itself as a change in the viscosity of the protoplasm and the supply of water to the tissues in the fumigated plants (Table 3).

As we can see from the data in Table 3, the total water content in the tissues of the fumigated plants was higher than that in the controls during the entire season. This was especially evident in the Muromskie variety.

The significant increase in water content of the tissues in the fumigated plants at the end of the season should be noted as an interesting fact. There is usually a decrease in the water content of plant tissues as the plants mature. Some authors correlate this with aging of the plant.

TABLE 1

The Effect of Presowing Fumigation on the Rate of Photosynthesis in Cucumbers (in g of material per 1 m^2 per hour)

Date of measurement	Controls			Fumigated		
	Accumulation	Loss	Total accumulation	Accumulation	Loss	Total accumulation
Variety Nerosimye						
June 1*	0.95	0.05	1.00	0.73	0.05	0.78
July 11	0.78	0.26	1.04	0.75	0.01	0.76
July 21	0.50	0.78	1.28	0.70	0.53	1.23
August 4	0.72	0.01	0.73	0.79	0.05	0.84
August 28 *	0.26	0.11	0.37	0.56	0.05	0.61
August 23*	0.38	0.05	0.43	0.66	0.13	0.79
Variety Muromskie						
July 1	0.72	0.25	0.97	0.37	0.20	0.57
July 11	0.77	0.32	1.09	0.79	0.15	0.94
July 21	0.56	0.21	0.77	0.74	0.05	0.79
August 4	0.75	0.19	0.94	0.62	0.20	0.82
August 23	0.35	0.05	0.40	0.24	0.22	0.46
August 28	0.19	0.05	0.24	0.16	0.28	0.44

TABLE 2

The Effect of Presowing Fumigation on the Process of Respiration in Cucumbers (in mg CO_2 released by the plant per hour per 1 g dry weight)

Date of measurement	Variety Muromskie		Variety Nerosimye	
	Controls	Fumigated	Controls	Fumigated
July 20	8.1	10.3	10.5	6.3
July 9	7.0	10.0	8.0	5.0
July 23	11.2	11.2	11.5	6.3
August 2	8.6	9.8	9.1	6.2
August 18	8.5	6.0	11.4	8.3
August 18	7.8	9.0	13.9	6.6

TABLE 3

The Effect of Presowing Fumigation on the Water Supply of the Tissues

Date of measurement	Variety Muromskie		Variety Nerosimye	
	Controls	Fumigated	Controls	Fumigated
July 11	82.0	83.0	80.8	80.0
July 21	83.2	83.0	80.0	81.0
August 4	81.3	83.0	79.5	81.5
August 23	84.6	86.3	84.6	83.0
August 28	83.5	89.9	83.2	86.1

Note: The water content of the plant tissues was determined by drying the plant to constant weight in an oven at a temperature of 105°.

The work of Alekseev [10] contains such examples. When the seeds were fumigated with carbon monoxide before sowing no decrease was observed. This fact must be considered as particularly important in the early maturing cucumbers (variety Muromskie); it makes it possible to obtain an increased yield in this variety until the end of the season.

The increased water content of the tissues in the fumigated plants is also in keeping with the increased rate of photosynthesis at the end of the season (see Table 1). All this indicates the great activity within the fumigated plants; we also noted an increase in yield at the end of the season.

Since great significance was attached to the viscosity of the protoplasm, we made a study of this using the plasmolytic method [11].

The data concerning the viscosity of protoplasm are given in Table 4.

From the data in Table 4 it appears that fumigation induced a marked change in the viscosity of protoplasm. The fumigated plants had a lowered protoplasmic viscosity during the entire season. Correlated with a decreased protoplasmic viscosity is an increase in the rate of the physiological processes, a more active relationship of the

*As in original. Publisher's note.

TABLE 4

The Effect of Presowing Fumigation on the Viscosity of Protoplasm (time in min for change from plasmolysis to turgor)

Date of measurement	Controls	Fumigated	Developmental stage of plant	Date of measurement	Controls	Fumigated	Developmental stage of plant
	Variety Muromskie				Variety Nerosimye		
July 10	39.0	32.0	Vegetative stage	July 11	33.5	32.0	Vegetative stage
July 24	13.5	6.5	Full flowering	July 25	36.8	6.8	Flowering
August 7	28.6	16.2	Fruiting	August 5	18.0	10.0	Beginning of fruiting
August 19	9.4	8.6					
August 25	14.4	12.0		August 21	8.6	6.8	Fruiting
				August 27	21.2	9.0	

organism with the surrounding environment, and a greater resistance of the plants to decreased temperatures. The experiments by Genkel and Margolina [12] indicate this.

This interrelationship of protoplasmic viscosity and the activity of the plant was also observed in our experiments on the rate of physiological processes. It was also evident from phenological data on the growth of the plant; this confirms the experiments by Genkel and Margolina [12].

An investigation of the physiological processes shows that under field conditions the factor of increased water content in the plant tissues as well as that of decreased protoplasmic viscosity is probably important. These physiological characteristics of the fumigated plants which accompany the increased activity of the plant should also indicate the yield of these plants.

Phenological observations on the fumigated plants showed that fumigation of the cucumber seeds with carbon monoxide did actually increase the overall activity of the plants. Fumigation induced an acceleration of the germination of the seeds. The fumigated plants germinated two days earlier than the controls and sprouted more uniformly than the controls. For example, on the twelfth day after planting the seeds in the soil, 55.6% of the total number of rows had completely sprouted plants in the plots with fumigated seeds of the Muromskie variety, whereas on the same day only 11.1% of the rows had completely sprouted on the control plots. On the same day 72% of the rows on the plots with fumigated seeds of the Nerosimye variety had completely sprouted plants, whereas on the control plots only 5.6% of the total number of rows had sprouted plants.

This increased growth was also observed during the vegetative growth of the plant which followed. The variation in vegetative growth of the fumigated and control plants was readily apparent from a glance at the plantings. On June 29th, i.e., on the 25th day after the seeds were planted in the soil, we counted the number of plants which had a stem five or more centimeters long and which had two or more regular leaves. Only 15.6% of the plants of the Nerosimye variety on the control plots were in this category, whereas 48.7% of the plants on the plots with fumigated seeds were in this group. The corresponding percentages for the Muromskie variety were 33 and 75. Therefore, it appears that the rate of growth in the fumigated plants was considerably greater than in the controls.

The fumigated plants bloomed earlier and more uniformly than the controls. On July 16th, 25.8% of the plants of the Muromskie variety of the control plots were in bloom, but on the plots with fumigated plants 77.6% of the plants were in bloom at this time. A similar condition was also observed with plants of the Nerosimye variety.

Increased growth was also evident from the dimensions of the leaf blade. The leaf blades on the fumigated plants were 21-27% greater than those on the controls (Table 5).

The dimensions of the leaf blades were obtained by a somewhat simplified method. In the summer, leaves from the control and experimental plants were picked (the second leaf from the top was picked) and placed in an herbarium press, after having been straightened carefully. In the winter the leaf area was determined from tracings of these leaves made on paper. The work was repeated 20 times.

TABLE 5

The Effect of Presowing Fumigation on the Dimensions of Cucumber Leaves

Variants of the experiment	Variety Muromskie		Variety Nerosimye	
	In cm^2	In % of the controls	In cm^2	In % of the controls
Controls	68.9	100	77.9	100
Fumigated	87.7	127	94.3	121

TABLE 6

The Effect of Presowing Fumigation on the Economic Properties of Cucumber Seeds

Variants of the experiment	Variety Muromskie		Variety Nerosimye	
	% Germination	Energy of sprouting	% Germination	Energy of sprouting
Controls	75.7	37	31.5	17.0
Fumigated	86.0	77.3	56.3	43.0

As was noted above (see Table 1), during their initial period of growth the fumigated plants exhibited a decrease in the rate of photosynthesis. However, Maksimov [13] attributed a greater significance to the rate of growth, which increased the overall working area of the plant, in increasing the yield than he did to the rate of photosynthesis. Fumigating cucumber seeds before sowing with this object in mind can be considered as an important procedure since it does increase the growth of cucumbers. The great activity of the plants was also evident from the fact that fumigated plants exhibited a greater resistance to unfavorable environmental factors than the controls; we attribute this to the decrease in their protoplasmic viscosity. For example, a count of the dead vines of the Muromskie variety showed that on August 25th 43.6% of the total number of the control vines on the plot were dead, whereas only 28% of the fumigated ones were dead; on August 30th, at the end of the experiment, the corresponding percentages were 73.3 and 59.3. Consequently, about half of the plants on the plots with fumigated plants were healthy, active plants; whereas 3/4 of the plants on the control plots were dead.

The total yield of cucumber fruits obtained in the given experiments was as follows: in the Muromskie variety the yield on the fumigated plants was 146% of the controls, in the Nerosimye variety it was 205.8% of the controls. The fumigated plants, consequently, had a yield of 1.5–2 times greater.

TABLE 7

The Effect of Presowing Fumigation of the Sex Reversal of Cucumber Plants

Date flowers counted	Controls		Fumigated	
	% Pistillate flowers	Ratio staminate flowers to pistillate ones	% Pistillate flowers	Ratio staminate flowers to pistillate ones
	Variety Muromskie			
2nd half of July	15	5.7:1	14	6.3:1
1st half of August	20	3.8:1	21	3.8:1
2nd half of August	35	1.8:1	31	2.2:1
Average for the season	23	3.3:1	21	3.8:1
	Variety Nerosimye			
2nd half of July	13	6.6:1	14	6.3:1
1st half of August	11.6	7.6:1	13	6.6:1
2nd half of August	9.4	9.6:1	8	11.0:1
Average for the season	10.8	8.2:1	11.6	7.7:1

It is very significant to note that presowing fumigation not only increased the yield in the fumigated plants but also induced earlier fruit formation; this made it possible to obtain both an earlier and a greater harvest. The yield in percentage of the controls (depending on the time of harvest) during the second half of July for the Muromskie variety was 164%, during the first half of August it was 217%, but during the second half

of August it was 130%; for the Nerosimye variety it was 622% during the first half of August and 158.8% during the second half of August.

Under conditions of vegetable crop rotation in the fields of the collective farm "Voskhod" the yield of fumigated cucumbers variety Nerosimye was 120.9% of the controls in 1952, and their greatest yield – 144.1% – was during the first period of harvest (the first half of August).

In 1955 the yield of cucumber plants variety Viaznikovskie in the greenhouses at the Botanical Garden of the Yaroslav Pedagogical Institute was 53% more than controls as a result of presowing fumigation of the seeds with carbon monoxide.

The increased activity of the fumigated plants was also expressed in the seed progeny. The percentage of germination as well as the rate of growth was greater for seeds obtained from fumigated plants than it was from those obtained from the controls (Table 6).

The data in Table 6 indicate that presowing fumigation of cucumber seeds with carbon monoxide induced great changes in the biology of the plants; these changes appeared in some degree even in the progeny of these plants.

The experiments of several authors [3-6, 8] on the fumigation of plants with carbon monoxide and carbon dioxide during the vegetative phase indicate that fumigation changes the rate of physiological processes and stimulates sex reversal of the plant in the female direction. As a result, there is significant formation of pistillate flowers.

We counted the number of flowers and made a note of the corresponding sex in the fumigated plants as well as in the controls; the results are given in Table 7.

The data from our experiments (Table 7) on the presowing fumigation of cucumber seeds shows that the marked sex reversal of cucumber plants which was observed, for example, by Minina [4] or which Edel'shtein mentioned [8], was not observed in this case.

The experiments by the authors mentioned above [4, 8] were performed on plants in the laboratory or in a greenhouse, and fumigation was done on mature plants immediately before flowering. It seems that the effect of fumigation on the plant immediately before flowering directs the physiological processes toward immediately stimulating the development of the female sex characteristics; this also has a great effect on the appearance of pistillate flowers.

In our experiments on the presowing influence of carbon monoxide, its influence on the plant was apparently obliterated by the influence of environmental factors.

SUMMARY

Carbon monoxide fumigation of cucumbers at an early period of development during the vernalization stage leads to metabolic changes in the fumigated plants which subsequently interact more actively with the environment. Fumigated plants exhibited more rapid growth, larger accumulation of vegetative substances, and, as a result of this an increase in the size of the leaf blade. It was also observed that flowering began earlier and proceeded more rapidly than otherwise.

The more energetic interaction between fumigated plants and their surroundings was also expressed in changes in the course of photosynthesis and modification of the colloids of the protoplasm – their hydrophilic nature became more prominent. The percentage of water in the tissues of fumigsted plants was higher and the viscosity of the protoplasm was lower than in the control plants.

Carbon monoxide fumigation at the initial period of plant life did not noticeably affect the sex of cucumber plants but the crop yield was from one and a half to twice that of the control plants.

Fumigation retarded the aging of plants by stimulating their growth and improving their vitality.

This method of presowing carbon monoxide fumigation treatment of cucumbers can be recommended for indoor as well as outdoor cultivation of cucumbers in the central regions of the Soviet Union. The value of this method lies in the fact that it does not retard growth processes as in the case when mature plants are fumigated.

By inducing viscosity variations in the protoplasm, fumigation at an early stage raises the plant's resistance to such an unfavorable factor of the environment as low temperature. This is especially essential for outdoor cucumber cultivation in the central and northern regions of the USSR.

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THE INFLUENCE OF NICOTINIC ACID ON THE GROWTH AND YIELD OF CERTAIN VEGETABLES

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Numerous investigations have shown that the vitamins in plants play an important role in metabolism and in assimilation of organic and mineral substances by the plants [1-7 and others].

However, there is no single point of view on the nature of the influence of single vitamins and their combinations upon the growth of higher plants. It has been found that a deficiency of nicotinic acid and thiamin causes retarded growth of isolated roots [7, 7a, 8]. Nicotinic acid does not have a substantial influence on the growth of roots in normally photosynthesizing plants. Some authors have deduced that supplying plants with nicotinic acid may have a result only at the time of their heterotrophic nutrition. However, other authors [9-12] have noted in a number of cases the positive effect of the vitamins thiamin, nicotinic acid, and their combinations with heteroauxin in accelerating the growth of shrubs and trees. Likewise, there is no single point of view on the nature of the influence of vitamins on growth of aerial plant parts. In conducting experiments with radish and cauliflower Minnum [13] noted that thiamin, riboflavin, and mixtures of them had no influence on these plants. Similar results have been obtained in experiments with Chrysanthemum [14, 15]. However, a number of authors have noted a positive effect of vitamins on the growth of aerial organs. Rakitin and Ovcharov [16] indicated that the introduction of nicotinic acid and adenine into the leaf by spraying produced more sturdy cotton plants: the plants had more leaves, sympodia and fruiting elements, they were distinguished by a more vigorous flowering and rapid maturity of the bolls and cotton yield was increased 15-20%. Analagous results were obtained later by other authors [17]. Positive results have also been observed by treatment of the Bukhara almond [8], grape [19] and lemon and soya plants [12]. Introduction of supplemental vitamins into the seeds of peas and wheat had a benefical effect on the growth of roots and aerial organs [6]. The pre-sowing treatment of carrot seeds with a 0.01% solution of nicotinic acid increased the growth processes and yield of carrots 30%.

It follows from this exceedingly brief and incomplete review that there is no single point of view on the role of supplementary vitamin nutrition in plant growth. However, this problem is exceedingly important both in theoretical and practical respects.

EXPERIMENTAL RESULTS

Our experiment was intended to explain the effect of nicotinic acid on the growth and yield of plants. As a rule the specified spring vegetables are raised on the ground indoors and increasing their yield is an economically important problem.

The experiments were conducted during 1954 and 1955. We are presenting the most characteristic data.

Hothouse Experiments with Seedling Onions

The onions were planted in a 35 × 55 cm box on April 4, 1954. The flat contained 13 kg of soil. Feritilizers in the following amounts were added to the soil before sowing: 0.25 g ammonium sulfate, 1 g of superphosphate, and 0.5 g of potassium chloride per kilogram of soil.

The experiment was repeated three times. Each box contained forty plants. The plants were sprayed

TABLE 1

The Effect of a Nicotinic Acid Spray on Vegetative Yield of Onion

Experimental treatments	Weight (g) of aerial portion of 40 plants			Yield increase (%) above control
	Replicate 1	Replicate 2	Average	
Control	126.0	118.8	122.4± 3.19	—
0.01% solution of nicotinic acid	192.0	186.0	189.0 ± 2.66	54.4

TABLE 2

The Effect Upon Lattuce Yield of Soaking the Seeds in Various Solutions

Experimental treatments	Weight (g) of plants from 1 m^2, (average of 3 replicates)	Yield increase (%) above control
Water (control)	610	—
0.01% solution of nicotinic acid	925	51
Solution nicotinic acid (0.01%) and heteroauxin (0.003%)	1150	88
Yeast extract	937	53

Note: Seeds treated with the preparation solutions germinated much earlier and more uniformly.

twice with a 0.01% solution of nicotinic acid at the time the leaves appeared and one week later. The results of the experiment are shown in Table 1.

As Table 1 shows, nicotinic acid increased the growth and yield of onion plants more than 50%.

A field experiment with brown onion and leafy lettuce was conducted in the botanical garden of the Lvov State University (LGU).

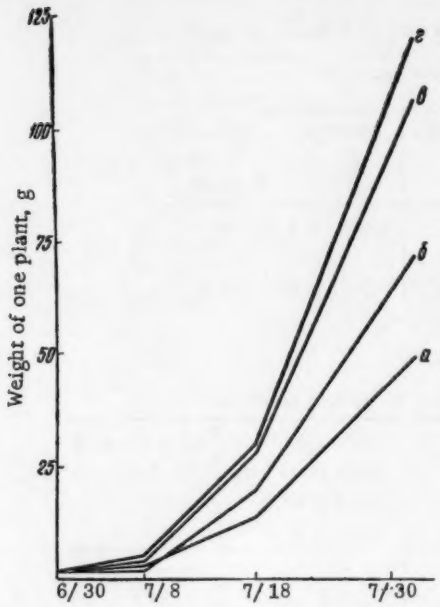
The soil was sandy. The plants were sown on May 15, 1954. Fertilizers were applied at the following rates before sowing: N-90, P_2O_5-125 and K_2O-90 kg/ha. The plots were 2 m^2. Three replicates were used. There were two hundred lettuce plants per plot. The onion plantings were not thinned. The seeds were soaked in water for 24 hours before sowing (control), in a 0.01% solution of nicotinic acid, in a yeast extract (60 g of yeast in 300 ml of water), and in a mixture of nicotinic acid and heteroauxin (100 mg of nicotinic acid and 30 mg of heteroauxin per liter). The data are shown in Table 2.

The data presented in Table 2 show the beneficial effect of seed treatment with solutions of the indicated substances upon lettuce growth and yield. Analogous results were obtained with onion and lettuce plants by spraying them ten days after the sprouts appeared with a 0.01% solution of nicotinic acid and also with a combination of 0.01% nicotinic acid and 0.003% heteroauxin at the rate of one liter per square meter. In this as in the other case the yield was increased 40-60%.

A positive effect was observed in our experiments only when the plants were treated at an early stage of development.

Experiments were performed with radish, lettuce, and tomatoes in 1955.

The experiments with radish were conducted in a cold frame at the B. Khmel'nitskii collective farm of the Lvov Region.



The effect of nicotinic acid on lettuce plant weight. a) Control; nicotinic acid concentrations: b) 0.1%; c) 0.001%; d) 0.01%.

Radish variety Rose-red with a white tip was sown on March 10. The plants were sprayed with a 0.01% solution of nicotinic acid 10 days after germination.

Immediately after spraying, the aerial part of the experimental plants became markedly more sturdy than the controls, which demonstrated the more rapid growth of the vitamin-treated plants. The harvest taken on April 24 showed that root yield in this case was noticeably increased. (Table 3).

The effect of different concentrations of nicotinic acid and the method of plant treatment on radish was investigated on open ground.

Seeds soaked in a 0.01% solution of nicotinic acid were used and at the beginning of root formation the plants were sprayed with 0.01% and 0.001% solutions of nicotinic acid. The experiments were conducted in the fields plots of the I. Franko Botanical Gardens (LGU). The soil was a chernozem. The seeds were sown in strips 8 meters long. The strips were spaced 14 cm. Seeds were sown August 24. Six kilograms of seed were sown per strip.

Total sugars were determined by the Berry micromethod and the vitamin C content by titration of Tilman's red. The experimental results are shown in Table 4 and 5.

TABLE 3

The Effect of Nicotinic Acid on Cold Frame Radish Yield

Experimental treatments	Root yield g/cold frame	Weight (g) of one root	Yield increase (%) above control
Control	800	8 ± 0.215	—
0.01% solution of nicotinic acid	2200	22 ± 1.187	175

TABLE 4

The Effect Upon Root Yield of Soaking Radish Seeds in a Nicotinic Acid Solution

Experimental treatments	Root yield g/strip	Yield increase (% above control)	Weight (g) of one root			Total sugars %
			Replicate 1	Replicate 2	Average	
Control	524	—	14.5 ± 0.97	14.9 ± 0.94	14.7 ± 1.78	1.21
0.01% nicotinic acid solution	1113	114.6	30.9 ± 0.93	32.2 ± 0.89	31.5 ± 0.58	2.18

As is seen from the data of Table 4, seed treatment with a nicotinic acid solution produces a marked increase in radish root weight (114% compared to the control).

TABLE 5

The Effect on Root Weight and Quality of Spraying Radish Leaves with a Nicotinic Acid Solution

Experimental treatments	Root moisture %	Weight (g) of one root (average of 3 replications)	Yield increase (%) above control	Total sugars (%)	Amount of vitamin C (mg %)
Control	94.17	11.56 ± 0.46	—	1.15	10.0
Nicotinic acid concentrations:					
0.001%	94.3	18.7 ± 0.42	61.5	—	—
0.01%	94.0	20.8 ± 0.14	73.7	2.06	11.02

The nicotinic acid also caused an increase in sugar content of the roots, which may be related to a change in metabolism and to an improved flow of nutrient materials into the roots of the experimental plants. Spraying the leaves with a nicotinic acid solution (Table 5) gave analogous results.

It is evident from Table 5 that a 0.01% solution of nicotinic acid had the greatest effect. According to preliminary analyses there was less lignin in the roots of radishes which were treated with nicotinic acid, and this is shown by their lesser lignification. The vascular tissue was more strongly developed than that of control plants, but the sclerenchyma was much more weakly developed.

The experiments with leaf lettuce* were conducted on the field plots of the Botanical Institute of the I. Franko LGU. Seeds were sown on June 14.

The soil was sandy. Fertilizers were applied at the following rates before sowing: N-90, P_2O_5-125, and K_2O-90 kg/ha. The plots were 4 m^2. Three replicates were used. One hundred fifty plants were left in each plot after the shoots appeared. The experimental plan was as follows: control (without spraying), and experimental, spraying with 0.1%, 0.01%, and 0.001% solutions of nicotinic acid. Spraying was done twice: on June 30, 16 days after germination (3-leaf stage) and on July 9 (5-leaf stage). Prior to the first spraying and 8-12 days after each spraying the following indices were determined: plant weight and height, leaf length, plant moisture content, and the amounts of vitamin C, chlorophyll, and carotin. Harvesting was done August 30.

The amount of chlorophyll was determined according to Getri. The amount of carotin was determined by a modification of Sapozhnikov's method. In each sample 100 juvenile or 10 mature plants were used. In the same way an experiment was conducted by soaking lettuce seeds in solutions of nicotinic acid. There was a visible effect of the treatment with nicotinic acid only seven days after the plants were sprayed.

The treated plants were easily distinguished from the controls by their greater height and leaf size. The experimental results are shown in Tables 6 and 7 and in the figure.

TABLE 6

The Effect on Lettuce Yield of Nicotinic Acid Spray Solutions

Experimental treatments	Yield kg/plot	Average weight (g) one plant	Yield increase (%) above control
Control	8.03 ± 0.10	53.5	—
Nicotinic acid concentrations: 0.1%	11.13 ± 0.10	74.2	38
0.01%	18.23 ± 0.33	121.5	125
0.001%	16.07 ± 0.09	107.1	100

*G. Zaretskaia and M. Bezpiatko, students of courses 4 and 5, participated in the work.

TABLE 7

The Effect of Nicotinic Acid Spray Solutions Upon the Vitamin C and Chlorophyll Content of Lettuce Leaves (mg %)

Experimental treatments	Vitamin C				Chlorophyll			
	6/30*	7/8	7/18	7/30	6/30	7/8	7/18	7/30
Control	23.6	26.8	45.3	54.2	58.8	60.2	61.9	69.7
Nicotinic acid concentrations								
0.1%	—	42.7	57.7	60.6	—	69.3	69.8	70.8
0.01%	—	33.8	69.2	72.7	—	66.0	71.2	79.0
0.001%	—	33.7	60.5	71.9	—	66.9	70.4	73.7

*Analysis before first spraying.

TABLE 8

The Effect of Nicotinic Acid on the Number of Tomato Fruits

Experimental treatments	Number of fruits on one plant (average of 3 replicates)	Yield increase (%) above control
Control	10.2 ± 0.94	—
Seeds soaked in 0.01% nicotinic acid solution	17.1 ± 1.27	68
Seeds soaked, plants sprayed with 0.01% nicotinic acid solution	24.0 ± 1.78	135
Plants sprayed with 0.01% nicotinic acid solution	18.0 ± 1.33	77

It is evident from the data of Table 6 that plant weight is appreciably increased by nicotinic acid spray solutions. For example, a 0.01% solution of nicotinic acid solution increased lettuce yield almost 2.5 times. Soaking lettuce seeds in nicotinic acid solutions also produced better plant growth. Treatment with 0.001% and 0.01% solutions increased plant height, weight of aerial organs and roots and, in the end, yield was increased 60-80%. In the same way the vitamin C and chlorophyll content was increased (Table 7). The carotin content was practically unchanged.

It should be noted also that the seeds treated with nicotinic acid sprouted earlier and more evenly. In the control plot (with a like amount of sown seed) 228 plants were produced, in the treatment with a 0.001% nicotinic acid solution 330 plants were produced, a 0.01% solution produced 337 plants, and a 0.1% solution produced 173 plants. From these data it appeared that a high concentration of nicotinic acid reduced the rate of development and depressed plant growth during the early vegetative period.

Experiments with Tomatoes. The effect of nicotinic acid solutions on the growth and fruiting of plants of tomato hybrid 172 x bison was investigated.

Seeds soaked in water and seeds soaked in a 0.01% solution of nicotinic acid were sown in a flat. The sowing was made on the plot on May 29. The soil was sandy. Fertilizer was added before planting at the following rates: N-90, P_2O_5-125 and K_2O-90 kg/ha. Fifteen days after sowing (June 13, 1955, buds appeared) the plants were sprayed with a 0.01% solution of nicotinic acid. Two hundred ml of solution was applied to every plant.

Experimental plan: control (seeds soaked in water, plants unsprayed); seeds soaked in a 0.01% solution of nicotinic acid; seeds soaked in nicotinic acid, plants sprayed with a 0.01% solution of nicotinic acid; seeds not soaked, plants sprayed with 0.01% solution of nicotinic acid. The experiment was replicated three times. Each

replicate contained 25 plants. Plant height, number and size of leaves, number of buds, flowering rhythm, the number of fruits per plant, and the fruit yield were determined. The determinations were made regularly for 7-10 days.

It was found during the progress of the investigation that plants grown from seeds soaked in a solution of nicotinic acid and plants sprayed with nicotinic acid were taller than the controls, and had a larger number and larger sized leaves. These plants had more buds and the ratio of flowers and buds was greater than in the control plants. Thus the nicotinic acid favorably influenced growth of the plants and caused a more active flowering and fruiting.

The number of fruits on each plant were counted when the fruit had formed but still had not ripened (Table 8).

The data of Table 8 show that the number of tomato fruits on a plant increases under the influence of nicotinic acid. The combination of seeds soaked in solutions of nicotinic acid and the plants sprayed with the solution had the greatest effect. The size of the fruits on the experimental plants was little different from that of the control. However, the amount of vitamin C and sugar in the fruit of plants treated with nicotinic acid was somewhat greater than in the control plants.

SUMMARY

The effect of nicotinic acid on the growth and yield of certain vegetable plants was investigated. The seeds were soaked in, and the plants sprayed with 0.1, 0.01 and 0.001% solutions of nicotinic acid. The experiments were conducted under field and indoor conditions in the Lvov region.

0.001 and 0.01% solutions of nicotinic acid improved plant growth and raised the sugar, vitamin C and chlorophyll content in leaves.

Treatment of the seeds and leaves with nicotinic acid solutions led to an appreciable increase in the yield of radish roots and the aerial mass of lettuce and onions, the increase being observed under field as well as indoor conditions.

The best effect was obtained with a 0.01% nicotinic acid solution. Higher concentrations retarded initial growth of the plants whereas lower concentrations had a smaller effect. Experiments with tomatoes indicated that best results are obtained if soaking of the seeds is followed by spraying of the plants with nicotinic acid. The plants in this case are much more vigorous and setting of fruits is enhanced.

The favorable effect of nicotinic acid was observed under various conditions of crop density.

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THE ABSORPTION OF MINERAL NITROGEN, PHOSPHORUS AND POTASSIUM DURING THE ONTOGENY OF BUCKWHEAT

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During different periods of ontogeny plants have different requirements for external environmental factors. Today much attention is being given to the study of plant requirements for light, moisture, and heat. The need for a certain light intensity after passing through the light stage, the so-called critical period, in relation to a decrease in light intensity and soil moisture has been shown for a whole series of plants [1-4]. A number of authors [5-12] have shown a differential plant requirement for mineral elements during the vegetative period.

Study of plant requirements for mineral elements has not always been linked with their stage of development. However, it is known that mineral nutrition conditions have a strong influence on the course of metabolic processes in the plants. Control of the conditions of mineral nutrition can alter the metabolism in a desired direction.

It is apparent from data in the literature [13, 14] that buckwheat is very sensitive to mineral nutrition conditions; however, the problem of absorption of mineral substances during various growth stages has not been studied sufficiently.

In this paper the absorption of nitrogen, phosphorous and potassium by buckwheat (varieties Aleksandrovskaia 134 and Amurskaia) is traced during different growth stages. By knowing the plant requirement for mineral nutrient elements during different growth stages, the most effective use can be made of fertilizers to ensure a large and stable yield.

We studied the absorption of mineral elements at the end of the light phase (treatment 1), during the period from the time the tip of the anthers was formed in the first flowers at the bottom of the inflorescence on the main tiller, up to the time a tetrad formed in the pollen mother cells of the same flower (treatment 2), after a tetrad had formed in the pollen mother cells (treatment 3), during the full flowering stage (treatment 4), during fruit formation (treatment 5) and while the fruits were turning brown (treatment 6).

In buckwheat the vernalization stage usually occurs in the fruit at germination or includes the first or second day of sprouting and continues for a total of 8-10 days.

In our investigations (1953-1955) we used the method proposed by Luk'ianov [15] for the study of buckwheat at the end of the light phase. According to this method the time when the plant does not react to long days is used as the end of the light phase.

METHODS

Buckwheat fruits were germinated in petri dishes and after sprouting they were transferred to a glass pan with water and later transferred to 930 ml of a full Knop's solution (supplemented by 0.2 mg of boron per vessel) with two plants per vessel.

The experiment was conducted with 30 vessels of which 10 were controls. The plants in the remaining vessels subjected to the same conditions were used for observation of points of development and samples were taken of dry weight accumulation. Distilled water was added daily to the nutrient solution in each vessel and

the mixture stirred up. The solution was changed at the end of every stage or developmental phase. At the time the solutions were changed two average samples were taken from which certain parallel determinations were made. The absorption of nitrogen, phosphorus and potassium was ascertained as the difference between their initial and final concentration in the solution. When the solutions were changed the pH was determined, and usually varied from 6.0 to 6.4. Study of the growing points—indicators of the completion of single phases of development—was conducted simultaneously with the determination of dry weight accumulation, at the time samples were taken for analysis when the solutions were changed.

The work was completed in the greenhouses of the Faculty of Plant Physiology LSKhI in Pushkin.

Analytical samples for determination of the absorption of mineral elements by Amurskaia buckwheat were taken at the following times: July 2 (end of light period), July 7 (middle of the plant growth period encompassing the second treatment), July 11 (end of the preceding period), July 19 (during the growing period corresponding to the third treatment), July 26 (time of general flowering), August 5 (time of fruit formation), August 18 (time of browning of the fruits).

Data are presented below on the absorption of mineral elements and dry weight accumulation by single phases of development and for the entire vegetative period (for the Amurskaia variety only, since the same regularity was observed in the Alexandrovskaia variety).

EXPERIMENTAL RESULTS

Absorption of the NO_3 ion. Nitrate absorption was determined colorometrically by the method of Granval-Liazhu. Nitrite and ammonia were never found by qualitative reactions in the analytical samples taken. In order to be sure of the absence of other forms of nitrogen except nitrate a total nitrogen determination was also made by the method of Kjeldahl and Iodl'-Bauer which determined all forms of nitrogen found in the solution.

The results of total and nitrate nitrogen determinations showed that nitrogen was found only in the nitrate form in the experimental solutions since the difference between the determinations fell within the limits of error.

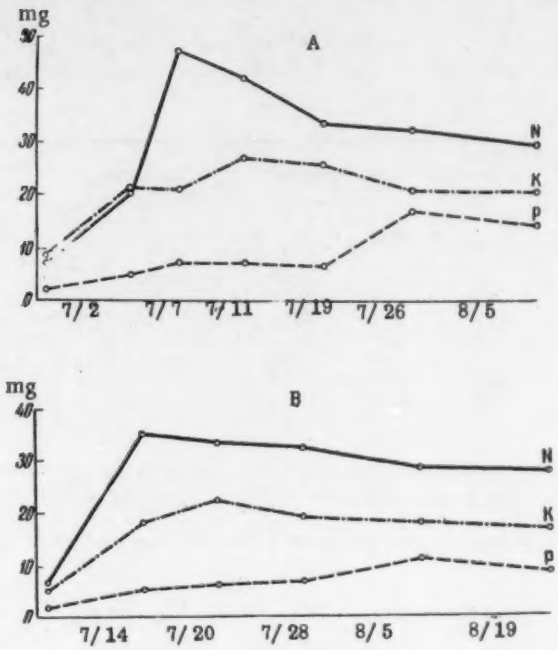
The experiments conducted showed that NO_3-ion absorption by buckwheat was irregular during different stages of development (see figure). During the light period stage of development (treatment 1) absorption was insignificant. Then it increased and reached a maximum during the period encompassed by treatment 2. Beyond this, absorption tended to decrease but continued to remain at a significant level. Absorption fell during the fruit-browning period, that is, at the end of vegetative growth. The greatest utilization of nitrogen occurred during the growth period encompassed by treatments 2 and 3. This is explained by the fact that during this time, as we established in the experiments in 1954, occurs the formation of all the inflorescences of the principal shoots and the laying down of the meristematic tubercles of the lateral tillers. Marked absorption of nitrogen during the subsequent phases is caused by the biological pecularity of buckwheat growth which results in the formation of vegetative and reproductive organs during almost the whole vegetative period.

The irregularity of nitrogen absorption during the vegetative period and the appearance of an absorption maximum during the spike-formation phase has been noted in analogous works connected with the study of nitrogen absorption by other crops—barley and wheat [9, 16]. The authors noted that the least amount of nitrogen was absorbed by plants during the light stage, which agrees completely with our data.

Absorption of phosphorus. The phosphorus determination was made colorometrically by the method of Fiske at the same time the NO_3 absorption was determined.

The literature has various data regarding the rate of phosphorus absorption by different plants during the vegetative period. Some authors [9, 11], in conducting their experiments, established that the greatest amount of phosphorus was absorbed during the first period of plant development. The results obtained by us in experiments with buckwheat showed that phosphorus was absorbed during all stages and phases of development and the maximum absorption occurred during the fruit-forming phase (see figure) and even during the period of fruit browning, the phosphorus requirement remained at a high level.

Intensive phosphorus absorption during the fruit-forming period, apparently, is explained by the fact that at this time stored nutrient substances are being laid down in the reproductive organs. Moreover, phosphorus in the form of adenosinetriphosphate participates in the complex synthesis processes going on intensively in the



Absolute absorption of nitrogen, phosphorus, and potassium by buckwheat per 24 hours (mg/100 plants). A) Amurskaia variety, B) Aleksandrovskaia 134.

fruits during their ripening period. An analogous regularity has been observed not only in buckwheat but in a number of other crops [5, 17].

Absorption of potassium. In our experiments potassium absorption was determined by the cobaltinitrite method. The curve of absolute potassium absorption had a somewhat different character in comparison with that of nitrogen and phosphorus (see figure).

The initial period of buckwheat development was characterized by an increasing vigor of potassium absorption. The absorption maximum occurred at the end of the growth period encompassed by treatment 3. After this the absorption rate somewhat decreased but continued to remain at a significant level.

A high plant requirement for potassium during this period has been noted in the works of Novikov, Sullerov, and Shapkina [18, 3, 19].

The experimental results showed that mineral elements studied are absorbed by plants in unequal amounts during different stages and phases of buckwheat development. The data on absorption was recalculated into milliequivalents for comparison of the absorption rate of separate elements.

The total absorption of NO_3', PO_4''' and K^+ in meq during the vegetative period and their quantitative ratios in the Amurskaia variety of buckwheat is seen in the following data:

Ion absorption, meq.			Ion ratios		
NO_3'	PO_4'''	K^+	$NO_3' : K^+$	$NO_3' : PO_4'''$	$PO_4''' : K^+$
118.4	51.9	30.0	3.94 : 1	2.28 : 1	1.78 : 1

It is evident from the data that the total absorption of NO_3' ion over the whole vegetative period was

TABLE 1

Average Absorption and the Ratio of Absorbed Ions During Various Periods in the Phenology of Buckwheat Variety Amurskaia (meq/10 plants/24 hours)

Experimental treatments	Duration (days) of the period in each exptl. treatment	Ion absorption			Ion ratios		
		NO_3'	PO_4'''	K^+	$NO_3':K^+$	$NO_3':PO_4'''$	$PO_4''':K^+$
1	11	0.50	0.20	0.20	2.50 : 1	2.50 : 1	1.00 : 1
2	9	3.35	0.66	0.53	6.36 : 1	5.07 : 1	1.25 : 1
3	8	3.00	0.65	0.71	4.22 : 1	4.60 : 1	0.91 : 1
4	7	2.40	0.66	0.67	3.58 : 1	3.63 : 1	0.98 : 1
5	10	2.32	1.64	0.54	4.29 : 1	1.44 : 1	3.04 : 1
6	13	2.10	1.40	0.53	4.00 : 1	1.50 : 1	2.64 : 1

almost four times as great as potassium absorption and 2.88 times as great as PO_4''' absorption. In the comparison of phosphorus and potassium absorption it appears that more PO_4''' ion is absorbed than K^+ ion.

The ratio in which mineral elements enter the plant during different periods in the life of the plant changed during the experiment (Table 1).

The great difference in ion absorption ratios for NO_3' and K^+, NO_3' and PO_4''' in treatment 2 attracts attention.

The ratio of PO_4''' to K^+ ion was markedly less during the entire development period than the ratio of NO_3' ion to K^+ and PO_4'''. An exception is the period of fruit formation (treatment 5) where the ratio is equal to 3 : 1.

It has been noted in the literature that a decreased dry matter accumulation during the flowering period was observed in plants which flower for brief periods. A decrease in dry matter was not observed in plants with protracted flowering period (to which group buckwheat belongs).

It is interesting to study how the dry matter accumulation and absorption of mineral elements changes during separate phenologic stages in buckwheat over one twenty-four-hour period. (Table 2).

TABLE 2

Absolute Dry Matter Accumulation and Uptake of Mineral Elements During Separate Plant Phenologic Periods Included in the Experimental Treatments (mg/24 hrs/10 Amurskaia buckwheat plants)

Indicators investigated	Experimental variant						
	1	2 (Middle of period)	2 (End of period)	3	4	5	6
Dry matter gain	91.0	186.0	238.0	828.0	925.0	1012.0	1267.6
Absorption							
N	7.1	20.4	48.0	43.0	34.0	32.5	29.6
P	2.1	5.0	7.0	7.0	6.9	17.0	14.6
K	8.4	20.8	20.9	27.3	26.4	21.4	21.1

Calculations show that the rate of dry matter accumulation and absorption of the elemtns of mineral nutrition do not coincide with the separate plant phenologic periods. The maximum accumulation of dry matter occurs during the period of fruit maturity (treatment 6). The greatest amount of nitrogen is absorbed during the period included in treatment 2, of potassium in treatment 3, but the greatest amount of phosphorus was absorbed in treatment 5 (during fruit formation).

TABLE 3

Absorption of Mineral Elements During Separate Plant Phenologic Periods (experimental treatments) (mg/100 mg of dry matter/24 hrs)

Elements	Experimental treatments						
	1	2 (Middle of period)	2 (End of period)	3	4	5	6
N	0.71	2.20	5.02	0.64	0.52	0.32	0.18
P	0.21	0.51	0.74	0.11	0.10	0.16	0.09
K	0.84	2.24	2.18	0.41	0.41	0.21	0.13

Coincidence of the accumulation maximum for nitrogen, phosphorus and potassium in the second treatment is evidently not accidental. This means that not only are nitrogen and phosphorus essential for formation of vegetative and reproductive organs but potassium is also essential for creation in the plant of conditions by which conversion of nitrogen and phosphorus which are more rapidly entering at this time can be accomplished.

It might be suggested that this period in the phenology of buckwheat is a sensitive and critical time in relation to the absorption of mineral elements.

SUMMARY

Maximum nitrogen absorption occurred at the time the anther heads formed in the first flowers of the lower inflorescence of the main shoot and continued until tetrads formed in the maternal cells of the same flowers (treatment 2).

Potassium absorption commenced at the time tetrads formed in the pollen mother cells of buckwheat and continued about 8 days; maximum phosphorus absorption coincided with the fruit formation stage.

The total NO_3' absorbed during the vegetative period was four times greater than the absorption of K^+ ions and twice the absorption of PO_4''' ions.

The ratio between the amount of absorbed ions depended on the age of the plant. The ratio of NO_3' to K^+ and NO_3' to PO_4''' was especially large during the period of treatment 2.

Dry matter accumulation increased continually but the gain was not uniform during various phenologic periods.

Mineral nutrient element absorption and accumulation of dry matter did not coincide during the phenologic periods in buckwheat.

Maximum mineral element absorption per 100 grams of dry matter occurred during the period of treatment 2.

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DISTRIBUTION OF RADIOACTIVE PHOSPHORUS (P^{32}) IN CERTAIN PLANTS IN CONNECTION WITH THE PHENOMENON OF POLARITY

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Investigations carried out in our laboratory have established a polar distribution of various organic substances in the plant organism [1-8]. It was interesting to clarify the nature of the concentration of mineral substances in the plant body. For this purpose, we used radioactive phosphorus.

There are a number of papers in the literature investigating the process of introduction and accumulation of this element in plant organisms [9-15]. However, we have found no papers in the literature concerning its polar distribution nor the distribution of other elements of mineral nutrition in the plant as single polar units [16].

For this reason, in 1955 we carried out an investigation of the distribution of the radioactive isotope of phosphorus in plants of cineraria (Cineraria hybrida Hort), hydrangea (Hydrangea hortensis Sm), the potato variety Kornea and oil-bearing perilla (Perilla ocymoides).

METHODS

The cineraria and hydrangea plants were grown in two kilogram pots with garden soil mixed with humus, and were used for the experiment in the stage of flower bud formation. P^{32} was introduced as labeled Na_2HPO_4 (at the rate of 10-12 million counts per minute per kg of air-dried soil). Radiophosphorus was added to a solution of KH_2PO_4 (carrier). The latter was added at the rate of 0.06 grams per kg of soil. The solution prepared in this way was poured into the pots with soil.

Throughout the experiment the shoots of the cineraria plants remained in normal condition.

In hydrangea plants, the main two-year shoot was cut at the base, and three young shoots developed at its base were used for the experiment. One of these one-year shoots remained in the normal state. The other was fastened with its top downward, and the third — in a horizontal position.

The potato and perilla plants were taken out of the soil at the stage of flower bud formation. The soil was washed off their roots, and they were placed in glass vessels with 50% Knop's solutions. P^{32} was introduced into Knop's solution as as labeled Na_2HPO_4, at the rate of 2.5 million counts per minute per liter.

After this, the potato and perilla plants were divided into four groups and for the next four days were kept: in darkness (first group); on a ten-hour day (second group); on the natural day (third group); and on continuous illumination (fourth group).

The radioactive phosphorus content in counts per minute was determined in the upper and lower zones of stem internodes, leaf blades, petioles, and roots.

The corresponding plant parts were ground in a mortar until a homogeneous mass was formed, of which 60 ± 5 mg were taken on aluminum sample plates 18 mm in diameter, and was spread in a thin layer as uniformly as possible over the entire surface of the plate. The count of the sample of plant tissue was made on

TABLE 1

Polar Distribution of Radioactive Phosphorus P^{32} in the Cineraria Plant Body
(In counts/minute per g wet weight; P^{32} was introduced into soil March 25, 1956)

No. of successive stem internodes, counting from the top	Zones of stem internodes, leaves, and root	March 26			March 28		
		Stem	Leaf		Stem	Leaf	
			Blade	Petiole		Blade	Petiole
Flower Bud					22035		
1	Tip	↑7294	↑5550	↑2808	↑12420	↑8000	↑5896
	Base	6484	5186	2510	9000	6302	5821
2	Tip	↑4600	↑8650	↓3549	↑7808	↑9412	↓4712
	Base	4520	7375	3823	6786	7710	4842
3	Tip	↑5000	↑8759	↓2526	↑7530	↑10520	↓5167
	Base	2754	6372	3034	7404	7520	6407
4	Tip	↑3150	—	—	↑9649	—	—
	Base	1173	—	—	6667	—	—
5	Tip	↑1932	—	—	↑9464	—	—
	Base	1423	—	—	7288	—	—
Root	Base	2613	—	—	11900	—	—
	Middle	2270	—	—	10964	—	—
	Tip	↓3060	—	—	↓13240	—	—

TABLE 2

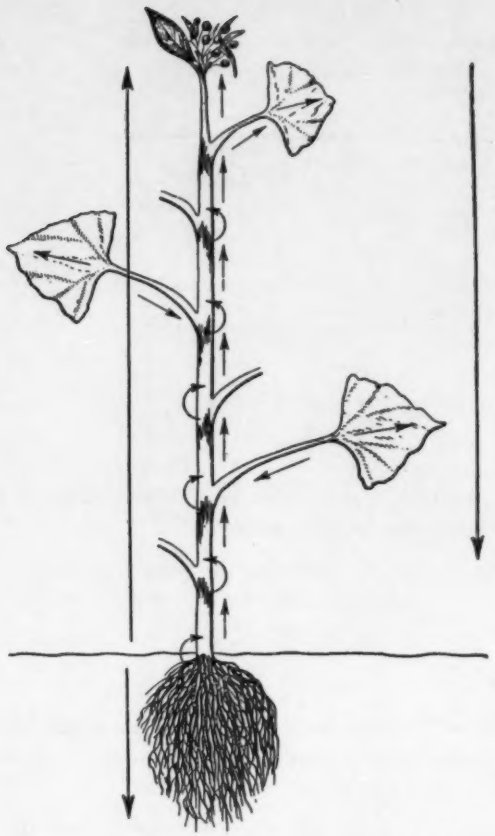
The Polar Distribution of Radioactive Phosphorus, P^{32}, in the One-Year-Old Shoot of the Hydrangea Plant in Various Positions with Relation to the Center of the Earth (P^{32} was introduced in soil on April 16. Experiment of 1955)

No. of successive stem internodes, counting from the top	Zones of stem internodes and leaves	In horizontal position		In inverted position		In normal position	
		Stem	Leaves	Stem	Leaves	Stem	Leaves
		Analysis April 18					
1	Tip	↑73	↑618	↓388	↓255	↑630	↑86
	Base	70	580	722	536	525	35
2	Tip	↑455	↑463	↑871	↑491	↑535	↑226
	Base	218	302	526	339	69	40
3	Tip	↑281	↑980	↓476	↑530	↑272	↑561
	Base	153	16	1326	517	245	277
4	Tip	↑274	↑627	↑1267	↑552	↑252	↑620
	Base	298	132	886	473	178	152
		Analysis April 25					
1	Tip	↑7000	↑8941	↑10966	↑7831	↑7107	↑2654
	Base	4946	4840	7815	6500	1964	1294
2	Tip	↑6816	↑6090	↑9929	↑16122	↓4720	↓2270
	Base	3700	5291	4539	8097	↓4871	↓3094
3	Tip	↑5490	↑10540	↑6367	↑11719	↑5357	↑3192
	Base	4083	6574	4980	8037	3700	2444
4	Tip	↑3760		↑6464		↑3750	↑4833
	Base	2040		5330		2575	3569

a counting device "B", using the face of the tube. The results obtained were recalculated in counts per minute per gram of the fresh wet plant material.

The experiment was done in duplicate.

As can be seen in Table 1, the content of radioactive phosphorus in the cineraria plant body is increased from the base of the root to the tips of the stem and root. The gradient of accumulation in the stem is step-like,



The diagram of the distribution of radioactive phosphorus in the cineraria plant body, as a single polar unit. The small straight arrows → show the direction of the smooth increase of P^{32} content in separate organs, and curved arrows ↷ show the gradation in its distribution from internode to internode along the stem.

Large arrows show the centrifugal nature of P^{32} concentration in the stem and root and the centripetal distribution along the leaf levels.

while in the root it is a gradual slope. In the leaf an even increase of P^{32} from the base of the tip is also observed, although its amount increases from the top to the base of the crown through leaves at successive levels.

From March 26 to March 28 the P^{32} content in cineraria was significantly increased, particularly in the root.

The diagram in the figure gives a visual concept of the polar distribution of radioactive phosphorus in the cineraria plant body as a single polar unit [16]. From the data of Table 2, it follows that the change in position of the hydrangea stem with relation to the center of the earth has an effect on the rate and nature of accumulation of radioactive phosphorus.

In the stem in the normal position, the P^{32} content increases in steps from internode to internode in both directions toward the third from the top internode, where its concentration reaches maximum value. In the leaves of the same shoot, on the contrary, the P^{32} content increases from the top to the lower levels.

In the stem fastened with the top downward, P^{32} is more concentrated in its lower zone. In the leaves of this stem, as in the stem in normal position, the phosphorus content is increased toward its lower levels.

Finally, in the stem in a horizontal position, there is also observed a retardation in translocation of radioactive phosphorus towards its tip, although it is not so sharply expressed. In the first days P^{32} is accumulated less in the tip of the stem than at its base. The leaves of the lower level of the stem in a horizontal position accumulate more phosphorus than do the upper leaves.

In stem internodes of all treatments taken separately, the P^{32} content is increased towards their tips, with the exception of two internodes of the inverted stem, which must be explained by its unnatural position.

In all experimental stems of hydrangea, again with the exception of the inverted stem, a gradation in P^{32} distribution from internode to internode is observed.

On April 25, i.e., ten days after phosphorus isotope introduction into the soil, the picture abruptly changes. The phosphorus content in hydrangea plants is sharply increased, and its maximum is accumulated in the inverted stem while the minimum is found in the normal stems. The same is observed in the leaves of these experimental treatments. However, the gradient of P^{32} concentration in stems and in leaves remained almost the same as it was on April 18. Thus, the deflection of the stem from its normal position retards radiophosphorus translocation along its tissues, and in connection with this, the polarity of its distribution in the plant body changes. It follows from this that, besides the adsorption forces [17], other forces act on the translocation of substances in plants, such as, for example, the force of gravitation, light, etc., [18-21]. Analysis of the stems and leaves of potato and perilla, which were kept under different conditions of illumination, shows

TABLE 3

The Effect of Day Length on Polar Distribution of Radioactive Phosphorus, P^{32}, In Shoots of Potato and Oily Perilla (P^{32} was introduced April 29; analysis was carried out July 2, 1955)

Zones of stem and levels of leaves	Darkness		Ten-hour day		Natural day		Continuous illumination	
	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
				Potato				
Upper	383	↑ 1618	17 078	↑ 21 500	34 133	↑ 26300	16 673	38 338
Middle	477	200	15 400	16 364	21 585	3200	30 982	28 220
Base	↓ 4783	73	↓ 17 346	12 491	↓ 36 836	3308	↓ 40 910	↓ 1241
				Perilla				
Upper	2167	200	6 531	↑ 15 564	↑ 21216	↑ 22 524	↑ 24 254	↑ 29 200
Middle	5477	6391	21 345	25 354	23795	15 954	20 819	9 400
Base	↓ 12217	↓ 5600	↓ 23 700	4 408	17588	8 083	18 593	13 061

that the maximum radioactive phosphorus accumulation is observed in plants on continuous illumination; it is less on the natural day, is still smaller on the ten-hour day, and is least in darkness (Table 3).

In general the P^{32} content in the stems of potato and perilla was increased from the upper pole to the lower, and along the levels of leaves toward the top. The stem of perilla on continuous illumination and natural day was an exception.

SUMMARY

1. These investigations revealed a polarity in distribution of radioactive phosphorus in plant organisms. The phosphorus isotope is concentrated in plant organs and metameres depending on their nature, the stage of development and the influence of environmental conditions.

2. In cineraria plants, phosphorus concentration is increased from the base of the root to the tips of stem and root. The same phenomenon is observed in the stems of hydrangea.

3. In the stems of potato and in perilla, in darkness and on a ten-hour day, phosphorus accumulation takes place in a reverse direction i.e., from the tips to the base of the root.

4. P^{32} content increases from the base to the tip of the cineraria leaf, and in the reverse direction in the petiole. In general, the same distribution of phosphorus is observed in leaf blades of hydrangea.

5. In the leaves of potato and perilla, the amount of radioactive phosphorus is increased from the lower to the upper levels. In cineraria and hydrangea it follows the reverse direction.

6. A step-like gradient phosphorus isotope distribution is observed in the stems of experimental plants and an even and smooth gradient is found in the root and leaves.

7. The use of the method of labeled atoms confirmed the polarity of distribution of substances in the plant body which we had established earlier by other methods.

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INVESTIGATION OF THE INFLUENCE OF THE MENTOR ON FORMATION OF HYBRID SEEDLINGS AND ITS DEPENDENCE ON THE POSITION OF THE GRAFT IN THE CROWN

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Developing I. V. Michurin's teachings concerning the mentor, Soviet investigators have tried to increase its effect by considering from which part of the tree to take cuttings and grafts, and how and on which trees to graft them [1, 2, 3]. Great attention has been given to the state of the hybrid seedling which is to be developed.

I. V. Michurin showed that hybrid seedlings in their development from the seed to the adult state change in morphological characteristics from the wild form to that of the cultivated variety.

Graded or metameric heterogeneity of biological, physiological, biochemical and morphological characteristics is represented by numerous data of the literature [4-12]. A large part of these papers is connected with study of the levels of leaves along the shoot, location of the fruit-bearing organs of annual plants, and metameric variety in quality of separate branches of perennial plants. Only an insignificant number of investigations have been devoted to the study of the level along the height of the tree crown.

The branches of the tree are formed in different environmental conditions. At different ages and developmental stages of the organism's life, they are influenced in their state by their distance from the root, and by microclimatic conditions – temperature, moisture, light.

The attention of investigators has long been drawn to the question of selection and quality of grafted and rooted cuttings. It was noted [3, 13, 14] that large variation in the planted material often occurred from the fact that the location of shoots in the crown was not taken into account, and therefore cuttings of various ages and states were taken for grafting. The heterogeneity of the seed generation of each separately-taken specimen is also explained by the multi-level location of fruit-bearing organs [14]. A practical conclusion from these works is that different levels of the crown give qualitatively different planting and seeding material.

The basic problem of the investigations which we carried out is the study of the effect of the levels of the mentor's crown on the degree of transmission of characteristics to the hybrid seedlings developed.

METHODS

As mentors we took twenty-five to thirty-year-old frost resistant scion-rooted apple trees of I. S. Gorshkov's selection (seedlings No. 2 and No. 37) and of S. F. Chernenko's selection (seedlings No. 28-53 and No. 1) and also forty-five-year-old trees of Antonovka, Grushovka, and Anis.

Scions of the apple Anis aportovoi were grafted onto the first four seedlings mentioned above; on the latter three varieties were grafted Anis aportovoi (Anis as mentor) and seedlings of the combination Borovinka × Renet Simirenko (Antonovka and Grushovka as mentors).

A seedling of wild frost-resistant pear was taken as a mentor for a five-year-old seedling of a pear of southern origin. The scions of Anis·aportovoi, Borovinka × Renet Simirenko and seedlings of the pear of southern origin were taken from the middle level of the crown and the middle part of the shoot. Scions were placed on the lower, middle and upper levels of the mentor's crown from the southern and southwestern sides.

TABLE 1

Sugars, Acidity, and Ascorbic Acid in the Fruits of Different Levels of the Crown of Apple Tree No. 2 and Its Grafts

Levels of the crown	Apple tree No. 2			Anis aportovoi						Apple tree No. 2	Anis aportovoi		Apple tree No. 2	Anis aportovoi	
	Mentor			Grafts			Scion roots			Mentor	Grafts	Scion roots	Mentor	Grafts	Scion roots
	Sugars (in per cent of wet weight)									Acidity in per cent of dry wt.			Ascorbic acid in mg percent of dry weight		
	Reducing	Sucrose	Sum	Reducing	Sucrose	Sum	Reducing	Sucrose	Sum						
Lower	8.48	2.67	11.15	7.64	1.19	8.83	7.32	1.12	8.44	1.55	1.82	2.46	10.9	9.3	5.0
Middle	8.16	2.03	10.19	7.24	1.07	8.31	7.54	1.28	8.82	1.66	1.82	2.36	10.5	8.4	8.9
Upper	9.60	2.94	12.54	9.08	1.54	10.62	7.96	1.54	9.50	1.18	1.71	2.14	18.1	10.4	9.5

The work was begun in 1952 and was carried out in experimental plots of the I. V. Michurin Central Genetic Laboratory.

We have observed that opening of the bud scales and opening of the buds begins earlier on the root suckers and on the lower levels, both in the trees of mentors and in grafts. This difference is sometimes as much as 2-3 days. We noted the same in laboratory conditions on cut branches in the winter-spring. A significant difference was observed in flowering. The lower levels of the crown begin to bloom earlier. In 1955, in seedling No. 2 the scions of Anis aportovoi, grafted on the lower, upper, and middle levels of the crown of the seedlings were the first to bear fruit. The upper level of seedling No. 2 has accelerated the fruit ripening of Anis aportovoi grafts by one and one-half to two weeks. In the fruits of upper levels and their grafts we found a greater percentage of sugar, lower acidity, and a greater amount of ascorbic acid. It should be noted that the fruits were harvested at the same stage of ripeness. (Table 1).

This phenomenon we explain by the difference in illumination and temperature conditions in upper and lower levels of the crown, as well as peculiarities of metabolism and water regime connected with age and stage changes, as well as with the distance from the root.

Many life processes in the plant depend to a considerable degree upon the state of water metabolism, among them the ability of the organism to endure unfavorable environmental conditions such as drought, wintering, etc. [11, 15-17]. The investigations of Zalenskii [6] showed that an increase of the degree of xeromorphy of leaves in the shoot is reflected in the quantitative increase of various anatomical-morphological characteristics. Not only structural, but also physiological-biochemical characteristics, change as one moves from the lower levels of leaves to the upper ones [6-8, 18]. Sisakian [17] established that the higher the level of the leaf on the shoot, the greater is its ability to endure water lack. From the data of Eremeev [18], it follows that in the leaves of fruit trees a repetition of physiological and anatomical indicators (physiological parallelism) is observed in the limits of every shoot of the crown. The lower leaves of every shoot are less drought-resistant. Unfortunately, this author does not give in his paper comparative data on leaves of the same age in shoots taken from different parts of the crown. According to the data of Guseva [9], there is much more biological similarity between upper and lower branches of different trees (of the same age and variety) than between upper and lower branches of the same tree.

We established that the water exchange of the apple trees and pear trees takes place in a different way in various levels of the crown. There are changes in the transpiration rate, in the suction tension, osmotic pressure, the state of water in leaves of the lower level as compared with the upper, the rate of respiration, sugar content in leaves. The change in transpiration rate changes the usual rate of physiological processes. The transpiration rate was determined by weighing samples of leaves on a torsion balance. A large loss of water by the leaves of the mentors and grafts was noticed in the lower level (Table 2).

TABLE 2

Transpiration Rate of Grafts of Anis aportovoi and Pear of Southern Origin on Various Levels of the Crown in Seedlings of Apple and Pear

Name of seedlings and date of analysis	Levels of the crown	In mg per g wet weight of leaves, average for 5 minutes	In g per m^2 leaf surface, average for 5 minutes	Name of seedlings and date of analysis	Levels of the crown	In mg per g wet weight of leaves, average for 5 minutes	In g per m^2 leaf surface, average for 5 minutes
Apple tree No. 2, July 3	Lower	41.2	9.49	Forest pear, Sept. 7	Root suckers	45.0	6.69
	Middle	32.7	6.38		Lower	43.3	6.38
	Upper	24.5	6.08		Middle	35.0	5.19
					Upper	28.3	5.63
Grafts of Anis aportovoi on apple tree No. 2, July 6				Grafts of southern pear on forest pear, Sept. 7			
	Lower	45.3	7.27		Root suckers	42.3	6.31
	Middle	43.4	5.05		Lower	64.0	9.49
	Upper	38.2	6.67		Middle	50.6	7.43
					Upper	35.0	5.93

Similar data were obtained with the other experimental objects. In 1955, we studied water loss by leaves from 7 A.M. to 5 P. M. It turned out that in the earlier period the leaves of upper levels transpired to a greater degree; in the hot part of the day, when the air becomes more dry, the upper leaves decrease their transpiration. On the average for ten hours, the greater water loss is found in the leaves of lower levels. In the hot hours of the day, the upper level leaves close their stomates, while in the lower leaves the stomates remain open.

The greatest transpiration rate was observed in the end phase of grand growth, then in the state of flowering, and the least transpiration rate was observed in the period of termination of vegetative growth.

The grafts on the lower level transpired in the same way as the level on which they were grafted. For example, the transpiration rate over 5 minutes of apple tree No. 28-53 was 3.85 g/ m^2 in the lower level, 2.84 in the middle level and 2.14 in the upper level; the graft of Anis aportovoi on apple tree No. 28-53 in the same five minutes transpired 6.30 g/ m^2 at the lower level, 6.15 in the middle level, and 5.86 in the upper level.

As was shown by Maksimov [11], at normal atmospheric humidity the osmotic pressure of the cell sap may

TABLE 3

Transpiration Rate of Experimental and Control Leaves at Various Levels of the Crown in Apple and Pear Seedlings* (Date of apple analysis, May20, pear– May 19)

Experimental treatments	Levels of the crown	Transpiration rate: mg per g wet weight for five minutes	Transpiration rate: g per m^2 leaf surface for five minutes	Experimental treatments	Levels of the crown	Transpiration rate: mg per g wet weight for five minutes	Transpiration rate: g per m^2 leaf surface for five minutes
	Apple tree No. 28-53				Forest pear		
Control	Lower	81.2	3.85	Control	Root suckers	149.2	7.12
	Middle	71.2	2.84		Middle	83.8	6.23
	Upper	56.1	2.14		Upper	117.1	6.23
Stomates covered by lanolin	Lower	18.9	0.92				
	Middle	23.4	0.92				
	Upper	19.9	0.93				
Cuticle coated with lanolin	Lower	83.8	2.66	Stomates covered by lanolin	Root suckers	38.5	0.48
	Middle	76.3	2.57		Middle	23.6	0.48
	Upper	66.9	1.90		Upper	26.2	0.48

* Control leaves were not coated with lanolin; in experimental ones, either cuticle or stomates were covered by lanolin.

TABLE 4

Water Content in Leaves of Various Crown Levels of the Seedlings of Apple and Pear

Kind of seedlings	Levels of the crown	May 26-28			July 8-12		
		Total	Free	Bound	Total	Free	Bound
Apple tree No. 2	Upper	67.9	48.5	19.4	56.5	28.9	27.6
	Middle	67.9	50.1	17.8	58.7	37.9	20.8
	Lower	68.65	51.7	16.8	59.0	34.9	24.1
Apple tree No. 37	Upper	74.0	52.0	22.0	64.1	23.4	40.7
	Middle	74.1	53.2	20.9	64.2	25.2	39.0
	Lower	75.0	54.7	20.3	61.5	22.8	38.7
Forest pear	Upper	65.4	19.5	46.2	52.2	36.2	16.0
	Middle	66.3	10.8	55.5	54.7	44.0	10.7
	Lower	66.4	30,2	36.2	55.1	47.5	7.6
	Root suckers	73.5	35.9	37.5	60.1	54.5	7.6

decrease the transpiration rate, which is confirmed by our data (Table 3).

However, osmotic pressure and stomate regulation alone are not sufficient to explain the higher transpiration of the leaves of the lower level as compared with the upper level. After coating the cuticle with lanolin, the transpiration rate was insignificantly changed. The leaves of the lower level transpired more rapidly than the leaves of the upper level, just as when the cuticle was not coated with lanolin. If the stomates are covered with lanolin, the transpiration rate sharply decreases, and the difference in the transpiration rate of leaves of various levels of the crown is decreased.

In our investigation, the actual age of the leaves is the same, but the leaves of the upper levels develop on branches which are older and are more mature, and therefore they have somewhat different properties than leaves on the lower levels. It is quite possible that the leaves of the lower levels, which have arisen at an earlier stage of tree development, having a greater free water content, use it more rapidly in the process of transpiration, which is confirmed by the data of Table 4.

TABLE 5

Osmotic Pressure and Suction Tension of the Leaves of Various Crown Levels in Seedlings of Apple and Grafts

Kind of seedlings	Levels	Osmotic pressure, in atm.	Suction tension, atm.
Apple tree No. 2	Lower	2.64	2.64
	Middle	8.13	6.70
	Upper	9.87	9.58
Apple tree No. 37 (mentor)	Lower	5.29	5.29
	Middle	9.87	6.70
	Upper	11.11	9.58
Grafts of Anis aportovoi on apple tree No. 37	Lower	6.05	5.31
	Middle	6.47	5.63
	Upper	8.10	7.12

Within the stem, the water is distributed unevenly: its content (in percent) in most varieties increases from the base to the top. Within the crown, however, the moisture of the wood reaches a maximum in the lower part and decreases towards the top of the tree [20]. Out data on the determination of total, free, and bound water in leaves of the same age snow that the greatest percentage of the total water present is contained in leaves of lower level, while the greatest percentage of bound water is found in leaves of upper levels. The same phenomenon was observed in one-year-old sections of branches at various levels (Table 4). In the winter period of 1954-1955, we studied the water content in buds and shoots. The data of these analyses showed that a greater percentage of water is contained in the branches and buds of the lower level than in branches and buds of the upper level. Thus, on February 17, in Renet bergamotnyi 48.3% water was found in the buds of the lower level and 43.3% in the buds of the upper level; in Pepin 4, 37% water was found in the buds of lower level and 34% the upper level; 40% in the shoots of the lower level and 36% in buds of those of the upper; in the shoots of the apple tree No. 37, 46% water was found in the upper level and 49% in the lower.

Evidently, the leaves of the upper levels are distinguished by greater resistence to water lack. According to Alekseev [15], the free water is most easily transpired from plants. Bound water is transpired with greater difficulty. It determines the stability of the protoplasmic colloids and the resistance of the organism to

TABLE 6

Sugar Content in Leaves of Various Crown Levels in Apple, Pear, and Their Grafts

Kinds of trees	Levels of the crown	May - June			July - August								
					Mentor			Grafts			Own roots		
		Reducing	Sucrose	Sum	Reducing	Sucrose	Sum	Reducing	Sucrose	Sum	Reducing	Sucrose	Sum
Apple tree No. 2 (mentor) and Anis aportovoi (graft and scion-root)	Upper	2.11	0.54	2.65	4.28	0.82	5.10	2.72	0.48	3.20	—	—	—
	Middle	—	—	—	3.76	0.94	4.70	—	—	—	2.06	0.82	—
	Lower	1.47	0.48	1.95	3.70	0.65	4.35	2.44	0.44	2.88	—	—	2.88
Forest pear (mentor) and seedling No. 2 (graft and scion-root)	Upper	3.29	1.03	4.32	2.85	0.64	3.49	3.06	0.71	3.77	—	—	—
	Middle	—	—	—	2.76	0.15	2.91	3.06	0.40	3.46	2.42	0.34	2.76
	Lower	3.23	0.65	3.88	2.68	0.15	2.83	2.70	0.19	2.89	—	—	—
Antonovka (mentor) and Borovinka x Renet Simirenko seedling (graft and scion-root)	Upper	—	—	—	3.17	0.27	3.44	3.14	0.75	3.86	—	—	—
	Middle	—	—	—	3.13	0.21	3.34	2.98	0.68	3.66	3.46	0.68	4.14
	Lower	—	—	—	2.84	0.08	2.92	2.93	0.59	3.52	—	—	—

unfavorable environmental conditions, particularly drought. In this connection, osmotic pressure, suction tension, and the xeromorphism of leaves of various crown levels in seedlings and grafts were investigated.

Determination of osmotic pressure was carried out plasmolytically with sucrose. The leaf was always taken from the same level; therefore leaves were of similar age. The suction tension, too, was determined in these leaves by means of a refractometer (Table 5).

Osmotic pressure and suction tension of the leaves of upper crown levels in trees is higher than in leaves of the lower levels. Our data on osmotic pressure and suction tension of leaves from various levels correspond to the data of a number of other authors [15, 18, 20]. The leaves of the upper levels of the grafts and the leaves of the upper levels of the crown have a higher osmotic pressure as in our experiment with apple. The higher osmotic pressure and suction tension favor water movement into the upper levels of the tree crown.

As was noticed earlier, the lower transpiration of the upper level as compared with the lower level may also be explained by an increased osmotic pressure of the leaves of the upper level and by their smaller free water content.

We have found significant differences in sugar accumulation; there are more sugars in the leaves and shoots of the upper levels, which, evidently, explains the higher osmotic pressure of the cell sap in the leaves of these levels (Table 6).

The leaves of the upper level are distinguished by smaller epidermal cells, a more extensive vascular network, and smaller stomates, but by a greater number of stomates per unit of leaf surface. In Table 7, the comparative dimensions of stomates of the upper, middle, and lower levels of crowns and their grafts are given.

From the data of Table 7, it is seen that the mentor has an effect on the structure of the leaves of the graft. Leaves of the grafts on the upper level are formed under more severe conditions of water regime; as do the leaves of upper level of the mentor, they begin to show characteristics of xeromorphism: smaller epidermal cells and smaller stomates, and a greater number of stomates per unit of leaf surface.

Zalenskii [6] and Maksimov [11], working with herbaceous plants, have noted that the upper leaves, in spite of their xeromorphic structure, possess a greater transpiration rate in spite of greater osmotic pressure. According to them, the increased transpiration of the upper leaf level favors a better water

TABLE 7

Stomates Dimensions in Grafts on Various Crown Levels of Seedlings of Apple and Pear in mm (Ocular 15x, Objective 40)

Kind of seedlings	Level of the crown	Length of stomates (1)	Width of stomates (2)	Product (1) x (2)
Apple tree No. 2	Lower	0.231	0.115	0.026
	Upper	0.205	0.108	0.022
Grafts of Anis aportovi on seedling of apple tree No. 2	Lower	0.241	0.129	0.031
	Upper	0.209	0.107	0.022
Forest pear	Lower	0.327	0.141	0.046
	Upper	0.269	0.123	0.033
Grafts of southern pear on forest pear	Lower	0.266	0.149	0.039
	Upper	0.264	0.143	0.037

supply in the upper parts of the plant. We have not observed such a phenomenon in our experiments. The upper levels of the seedling crown, being in the more difficult conditions of water regime, use water more economically, and absorb it by the development of greater osmotic pressure and increased suction tension.

The more rapid respiration of leaves in the upper level evidently may favor better water absorption since it is known that, in addition to energy processes, respiration is also connected with growth processes. Respiration serves for supporting the protoplasmic structure in a viable state: it is necessary for absorption of mineral salts and water; it also regulates the process of fruit ripening etc.

We determined the rate of respiration (Table 8) by a gasometric method using a Warburg apparatus, and also by the method of Boysen-Jensen. The data obtained by the two methods are in complete correspondence.

TABLE 8

Respiration Rate of Leaves from Various Crown Levels in Apple Seedlings

Kinds of plants	Levels of the crown	Amount of oxygen absorbed			
		In mm^3/g of wet weight of leaves		In percent of the upper level	
		June 21-22	July 22-28	July 21-22	July 22-28
Apple tree No. 37	Upper	307.6	236.0	100.0	100.0
	Middle	301.0	175.3	97.8	74.2
	Lower	256.4	130.0	83.3	55.0
Apple tree No. 2	Upper	287.2	192.9	100.0	100.0
	Middle	184.8	167.7	64.3	86.9
	Lower	195.8	149.6	69.7	72.4

As can be seen from the data in Table 8, in June, when growth of branches is still observed in trees, the respiration rate is higher as compared with the end of July, when their growth is already terminated. The leaves of the upper level respire considerably more rapidly than do the leaves of the lower level. The same results were obtained with other experimental trees. Thus, for example, on July 28 the respiration rate of pear leaves in the upper level was 123.4 mm^3 of oxygen per g of wet weight, and in the lower level – 72.5 mm^3 of oxygen per g of wet weight of leaves.

For diagnosis of drought resistance and ability to withstand water lack we used the starch test – desiccator method, proposed by Genkel [21, 22]. The results of our analyses showed that the greatest amount of starch is found in the leaves of the upper level after they are held in a desiccator above sulfuric acid for 1.5 hours. The leaves of the upper level also have a greater capacity to endure water lack.

On the basis of these investigations, we conclude that the leaves and shoots of the upper levels of tree crowns are more adapted to the environmental conditions, and are more drought resistant.

SUMMARY

1. The morphological and physiological nature of various parts of the crown differ. This qualitative difference materially influences grafts. Water conditions in the leaves of top level grafts are more stringent and the grafts, just as the top levels of the mentor crown, form more xeromorphic leaves.

2. As compared to graminaceous plants the more xeromorphic organization of the top level leaves does not lead to an increase in the intensity of transpiration; on the contrary, a lower rate of transpiration is observed due to increase of the amount of bound water and enhancement of osmotic pressure.

3. The physiological nature of the leaves of the graft and the leaves of the level where the graft was inserted are very similar. Leaves of shoots of the top part of the crown are more drought resistant, and the transpiration rate in them is much smaller during the warmest hours of the day.

4. The top, middle and lower levels of the crowns of the mentors exert different influences on hybrid seedlings. This is due to differences in metabolism, water conditions and also to different conditions at various heights of the tree crown. One may suppose that more drought-resistant organisms will be developed in the upper levels of the crown.

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THE SITE OF CATECHIN SYNTHESIS IN THE TEA PLANT

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In spite of numerous investigations, the problem concerning the course and site of tannin synthesis in plants cannot yet be considered solved. It is known that many substances of secondary origin are synthesized in the roots. Shmuk, Smirnov, and Il'in [1] showed that nicotine synthesis occurs in tobacco roots, and Mothes and his co-workers found that the roots are the primary site of allantoin [2, 3] and citrulline [4] synthesis. Later Mothes and Kala [5] discovered that the roots of Atropa belladonna cultivated in isolation were capable of synthesizing tannins related to coumarin, umbelliferone (7-oxycoumarin) and scopoletin (6-methoxy-7-oxycoumarin), derivatives of phenylpropane which are similar to catechin. Isolated roots of Arachis hypogaea synthesize flavanone, a substance even more closely related to tannins [6].

On the other hand, Zaprometov [7] observed an increase in the tannin content of young tea leaves separated from the plant after they had been kept at a temperature of + 5° for 20-40 hours. According to data from Kursanov and Kriukova [8], within 20 hours excised tea leaves synthesized up to 25 mg of tannins per 1 g dry leaf weight. The infiltration of tea leaves with sucrose, glucose-1-phosphate, fructose, and glucose [9] also resulted in the accumulation of tannins of the catechin type.

In a detailed investigation Nick [10] concluded that in snakeweed (Polygonum bistora L.) tannins are synthesized independently in the leaves as well as in the roots, the flavones related to the tannins are synthesized only in the leaves, therefore light is necessary for the synthesis of flavones. While studying leucoanthocyanins, which may be considered as precursors of tannins and flavones, Hillis [11] found that in eucalyptus the leaf is the most likely site of their primary synthesis.

It has been shown earlier by the author [12], that catechins – these are the most common representatives of tannins – are synthesized vigorously during the early stages of tea seed germination. Furthermore, 60-day-old etiolated seedlings did not differ noticeably from normal seedlings in respect to the composition and quantity of catechins present; this gives support to the nonphotosynthetic course of catechin synthesis. However, such a result, apparently, may also have been due to the fact that the catechins in the 60-day-old seedlings had been formed to a considerable degree from stored products which entered from the cotyledons.

The isotope method was used in the work described below in order to try to locate the site of catechin synthesis. The tea plant was used as experimental material because the composition of its tannic compounds had been studied previously [13].

EXPERIMENTAL PLAN

Three-year-old tea plants of a Georgian variety which had been raised in the greenhouse in containers with peat moss were used for the experiment. Three plants as nearly alike as possible in development were placed in a plexiglass chamber which had a 360 l capacity (the type described by Merenova [14]). Plants from two other chambers which served as the controls were freed of soil, the roots were cut off under water, and the aerial parts with cut ends immersed in a glass of water were placed in this chamber. Two small cups, each of which contained up to 1.25 mc $BaC^{14}O_3$ and the corresponding amount of $BaCO_3$ necessary to generate a concentration of CO_2 within the chamber up to a volume of 0.1%, were placed on the shelf within. Two dropper funnels containing 2 N H_2SO_4 were placed over the small cups. After an air-tight seal had been obtained, a

very weak vacuum was produced within the chamber by using a water-jet pump; and following this the carbon dioxide from the first portion of $BaC^{14}O_3$ was displaced by the addition of 2 N H_2SO_4 from the dropper funnel. After 48 hours the second portion of $C^{14}O_2$ was introduced into the chamber in a similar fashion. The experiment was performed in Moscow from July 20 to 24 during cool weather (air temperature during the day was 18-21°, at night 8-11°) with variable degrees of cloudiness and occasional light rains. During the day when the weather cleared a protective screen consisting of three layers of gauze was placed over the chamber. After 92 hours air was passed through the chamber with the aid of a water-jet pump; the remaining $C^{14}O_2$ was absorbed with alkali. The chamber was then opened and detached parts of the plants were fixed in boiling 96% alcohol.

The following were selected for study: 1) young trifoliate shoots, normal as well as suppressed ones (shoots without developing buds), 2) mature leaves (fifth and sixth), 3) bark from woody parts of the stem, 4) wood situated under the bark taken for study, 5) roots (in three experimental plants). The remaining leaves, nonwoody stems, and subterranean parts of the stem were not analyzed.

METHODS

The fixed specimens were extracted once with ethanol. The combined alcohol extracts were condensed to a small volume under vacuum, and the remaining extract was dissolved in warm water. The residue of resins and pigments was filtered off. In order to remove the caffeine, the water solution was rinsed twice with chloroform (up to $^1/_6$ of the volume of water layer) the chloroform residue was removed under vacuum, and the solution was extracted six times with freshly distilled ethyl acetate ($^1/_6$ the volume of the water layer). The combined

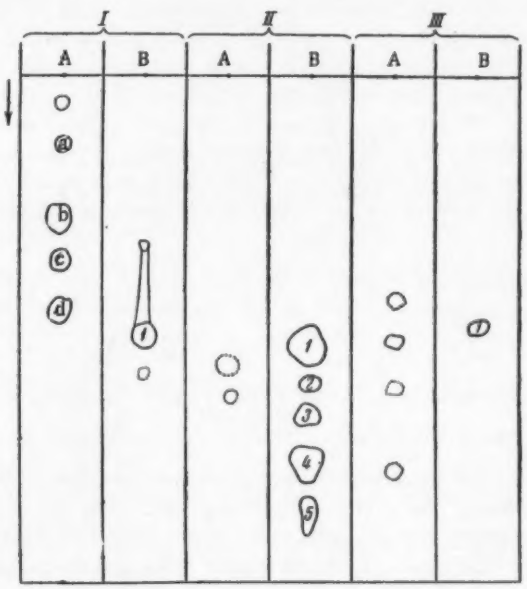

Fig. 1. Chromatograms of fractions obtained by rinsing the water extract from the mature tea leaves with ethyl acetate (diagrammatic). I) Water residue; II) ethyl acetate extracts; III) wash waters. A) Developed with carbamide; B) developed with vanillin. Chromatographic paper No. 3 experimentally produced TsNIIB (manually operated reflux); solvent – n-butanol: CH_3COOH: H_2O (40 : 12 : 28) 1) l-epigallocatechin; 2) d, l-gallocatechin; 3) l-epicatechin and d,l-catechin; 4) l-epigallocatechingallate and l-gallocatechingallate; 5) l-epicatechingallate; a) raffinose; b) sucrose; c) glucose; d) fructose.

ethyl acetate extracts were washed twice in a small volume of water ($^1/_{15}$ the volume of the ethyl acetate extract). The fractions obtained were studied with the use of paper chromatography. The catechins were developed with a 1% solution of vanillin in concentrated HCl [15], the sugar with a carbamide solution [16].

The schematic diagrams of chromatograms of the water residue after extraction with ethyl acetate, the ethyl acetate extract itself, and the wash water from mature leaf specimens (fifth or sixth from the top) are presented in Fig. 1.

It is evident from Fig. 1 that sugars are present in the water residue, a portion of l-epigallocatechin, traces of d, l-gallocatechin, and an unidentified substance with a low R_f value, which stained with vanillin and was present in a very small amount. A whole complex of catechins was found in the ethyl acetate extract, as well as traces of two substances which stained with carbamide. A portion of l-epigallocatechin and a very small amount of substance which stained with carbamide was present in the wash waters. Judging by the intense staining of the spots, about half of the total amount of l-epigallocatechin and practically all the remaining catechins were in the ethyl acetate extract. When the chromatograms of the ethyl acetate extract were developed with ninhydrin and bromphenol blue no amino acids or strongly dissociating organic acids were detected.

The treatment described above permits isolation of part of the catechins.

A quantitative determination of catechins after a chromatographic separation of the ethyl acetate fraction on paper has previously been made using the method described [17].

In order to identify the sugars, after the water residue had been extracted with ethyl acetate it was combined with the wash waters and passed successively through columns with cation carrier KY-2 (in the H^+ form) and anion carrier PE-9 (in the OH^- form). The residue, which usually had a neutral reaction, was evaporated under vacuum to a small volume and used for paper chromatographic separation [the solvent was n-butanol: CH_3COOH: H_2O (40 : 10 : 50)], the developers were carbamide, naphthoresorcinol and p-anisedine). Chromatograms of the sugars in the young shoots and mature leaves contained fructose, glucose, sucrose, and a small amount of raffinose. Checking this chromatogram on a densitometer with a dial indicator revealed the absence of any spots with high radioactivity. Strips of chromatograms (usually 7-8 spots were placed side by side on one sheet of paper) corresponding to the position of one or another sugar were eluted with water, and samples were taken from the solution obtained in order to make a quantitative test with anthranone [18] and to determine the radioactivity on brass plates.

The activity of the specimens was determined from an extremely thin layer, using a dial indicator and an assembly of Type B. All the figures were obtained without conversion, i.e., they represented an indication of the electromechanical calculator.

DISTRIBUTION OF RADIOACTIVITY IN VARIOUS PARTS OF THE PLANTS

The results of radioactivity determinations of the initial alcohol extract are given in Table 1.

From Table 1 it is evident that the principal activity was centered in the young three-leaved shoots and the mature (5th-6th) leaves (84.4% of the total activity of the shoot + fifth and sixth leaves + bark + wood in plants with roots, and 32.5% in plants without roots). The roots of the experimental plants had a similar radioactivity but it was considerably less, especially if one considers the data pertaining to the unit of fresh weight of the specimens (Table 2).

It is evident from the data in Table 2 that the removal of the roots did not retard the photosynthetic incorporation of $C^{14}O_2$ by the tea plant. Plants without roots even exhibited a somehwat greater radioactivity in this case when calculated on the basis of 1 g fresh weight of tissue. This variation was especially great for young shoots. It can be explained by the movement of labeled photosynthates into the root system in the intact plants. The greatly increased radioactivity of the bark in plants without roots is of interest. It consisted of 5.89×10^6 counts/min per 1 g fresh weight as compared to 2.00×10^6 counts/min per 1 g fresh weight in the intact plants. The same sort of thing can also be seen in Table 1 where part of the activity of the alcohol fraction from the bark was higher in plants without roots as compared to the intact plant at the expense of the activity of the shoot. From this one may conclude that radioactive photosynthates are translocated along the stem and accumulate in the bark of plants from which the roots have been removed.

In order to find out just how closely the data presented above could be related primarily to the more closely

TABLE 1

Total Activity of Alcohol Extracts (in counts/min)

Plant part from which extract was obtained	Plants with roots		Plants without roots	
	Activity in counts/min	Activity in % of total: shoot + fifth-sixth leaves + bark + wood	Activity in counts/min	Activity in % total: shoot + fifth-sixth leaves + bark + wood
Young three-leaved shoots	390×10^6	52.24 } 84.4	13.8×10^6	49.85 } 82.5
Fifth-sixth leaves	240×10^6	32.15 }	89.5×10^6	32.61 }
Bark	63.1×10^6	8.45	28.2×10^6	10.28
Wood	53.3×10^6	7.14	19.9×10^6	7.25
Root	81.6×10^6	—	—	

TABLE 2

Radioactivity of the Initial Alcohol Extracts, in Relation to 1 g Fresh Weight of Tissue (in counts/min)

Plant parts	Plants with roots	Plants without roots
Young three-leaved shoots	11.03×10^6	29.7×10^6
Fifth-sixth leaves	3.98×10^6	4.22×10^6
Bark	2.00×10^6	5.89×10^6
Wood	0.82×10^6	1.01×10^6
Root	0.64×10^6	—

related products of photosynthesis, especially the sugars, instead of the catechins — products of secondary origin — it was necessary to make a more detailed study of the radioactivity of certain groups of compounds. With this object in mind the activity of the fraction obtained after rinsing the water extract with ethyl acetate (see method) was studied. The results obtained are given in Table 3.

Actually, the specific radioactivity of the fraction of catechins in the mature leaves was only about 3%, as compared with the specific activity of the water extract after its extraction with ethyl acetate, in the intact plants as well as in plants with the roots removed (Table 3). At that time the fraction of catechins in the young shoots exhibited a high radioactivity (6980 counts/min/mg, which constituted about 28.5% of the activity in the water residue).

More complete data about the catechin fraction obtained from various parts of the experimental and control plants are given in Table 4.

From Table 4 it is evident that the specific activity of the catechin fraction from young shoots was many times greater than in other parts of the plant. Even in the mature leaves, in spite of the occurrence of photosynthesis, the radioactivity of the catechin fraction was of the same order as that in non-photosynthesizing organs. It is apparent that the formation of catechins in the roots can not be of any great significance in the tea bush since plants with their roots removed continued to synthesize catechins in approximately the same amounts as intact plants.

TABLE 3

Specific Activity of the Fraction Obtained after Rinsing the Water Extract with Ethyl Acetate (in counts/min/mg)

Fraction	Mature leaves (plants with roots)	Mature leaves (plants without roots)	Young shoots (plants without roots)
Water extract after rinsing with chloroform and ethyl acetate*	11,900	12,900	24500
Ethyl acetate extract after rinsing with water (catechin fraction)	396	407	6980
Rinse waters*	545	698	Not analyzed

* Without purification with ion exchange.

TABLE 4

Specific Activity of the Catechin Fraction (Ethyl Acetate Extract) from Various Parts of the Tea Plant (in counts/ min/ mg)

Plant part	Plants with roots	Plants without roots
Young shoots	9800	6980
Mature Leaves	396	407
Bark	465	560
Wood	435	610
Root	590	—

Not all the principal possibilities of catechin synthesis in the roots of the tea plant were studied. This can be definitely checked only with special experiments.

The primary site of catechin synthesis may be either the young three-leaved shoots or the mature leaves, whereupon, since the specific radioactivity of catechins from the mature leaves is not great, the catechins formed in this case must move rapidly into the new shoots.

FIXATION OF $C^{14}O_2$ BY EXCISED MATURE LEAVES AND YOUNG SHOOTS

In order to answer the question whether labeled catechins are synthesized directly in the young shoots, or whether they enter these from the mature leaves, in a supplementary experiment, labeled C^{14} in the carbon dioxide was introduced photosynthetically into the fifth or sixth leaves or into three-leaved shoots isolated from the plant.

The specific radioactivity of the catechin fraction (ethyl acetate extract) in mature leaves and young shoots isolated from the plant after a three-hour exposure to $C^{14}O_2$ (in counts/ min/ mg) was as follows:

Plant part	Specific activity
Young three-leaved shoots	5400
Mature leaves (fifth-sixth)	160

The experiment was performed on 10 shoots and a corresponding number (20) of mature leaves in a 1 liter flask. At the beginning of the experiment 0.6 mc of $C^{14}O_2$ was introduced into the flask.

As one can see from the data, the distribution of radioactivity in the ethyl acetate fraction was the same in excised leaves and shoots as it was in the intact plant. Mature leaves synthesized practically no labeled catechins, whereas the synthesis of catechins in the young shoots occurred very actively. On this basis it must admitted that the accumulation of labeled catechins in the young shoots does not occur as a result of their translocation from mature leaves – labeled catechins are synthesized in the shoots themselves. Hence, the primary sites of catechin formation in the tea plant are the young shoots.

RADIOACTIVITY OF INDIVIDUAL CATECHINS AND SUGARS IN YOUNG SHOOTS AND MATURE LEAVES

Experiment with Intact Plants

In order to make a preliminary determination of radioactivity in the catechins and sugars, the chromatograms were checked with a densitometer with a dial indicator. Figures 2 and 3 are of curves representing the radioactivity of chromatograms from the initial alcohol extracts and the ethyl acetate fraction (catechins) obtained from young shoots and mature leaves.

Inasmuch as the primary goal was the best separation of catechins, as a solvent we used a mixture of n-butanol: CH_3COOH: H_2O (30 : 12 : 28) was used; this was more suitable for the catechins, but did not give a sharp separation of sugars. For this reason the chromatograms for sucrose, glucose, and, in case of the mature leaves, for fructose, given in Fig. 2 and 3 were combined into one spot when checked with the densitometer.

The chromatograms given in Figs. 2 and 3 show that sugars had a high radioactivity, both in the young shoots as well as the mature leaves. At that time the catechins had a high activity only in the young shoots; this agrees with the data in Tables 3 and 4.

Individual catechins in the ethyl acetate fraction of the bark, the wood, and the roots also had a very low radioactivity, similar to those in the mature leaves, and were distinguished with difficulty when the corresponding chromatograms were checked with the densitometer. Nevertheless, the very fact that labeled catechins did exist in the bark and wood of the stem, as well as in the roots, is evidence of their mobility.

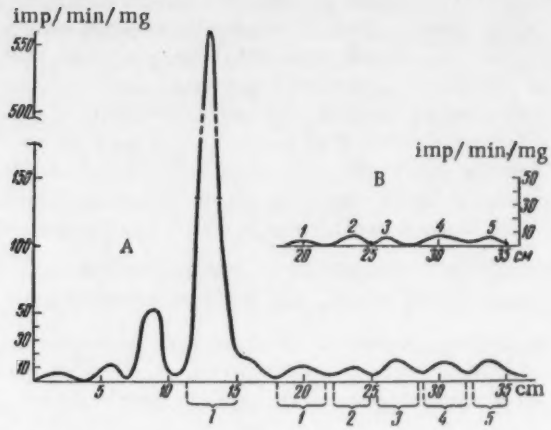

Fig. 2. Chromatograms of the initial alcohol extract and the corresponding catechin fraction from mature leaves (activity checked on a dial indicator densitometer). A) Initial alcohol extract; B) ethyl acetate (catechin) fraction. I) Sucrose, glucose, and fructose; 1) l-epigallocatechin; 2) d,l-gallocatechin; 3) l-epicatechin; 4) l-epigallocatechingallate + l-gallocatechingallate; 5) l-epicatechingallate.

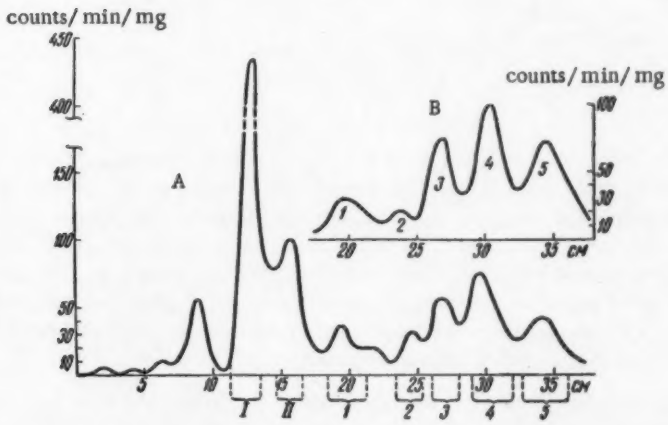

Fig. 3. Chromatograms of the initial alcohol extract and the corresponding catechin fractions from young three-leaved shoots (activity checked with a dial indicator densitometer). A) Initial alcohol extract; B) ethyl acetate (catechin) fraction. I) Sucrose and glucose; II) fructose; 1) l-epigallocatechin; 2) d,l-gallocatechin; 3) l-epicatechin, d,l-catechin; 4) l-epigallocatechingallate l-gallocatechingallate; 5) l-epicatechingallate.

Having been formed in the leaves as the result of photosynthesis, the catechins are capable of moving throughout the plant, and there is the possibility that a significant accumulation of tannins in the roots of a tea plant [19] occurs as the result of translocation of catechins from the leaves. Chromatograms of ethyl acetate extracts of

bark and wood primarily contained l-epicatechin, d,l-catechin, and d,l-gallocatechin. In these tissues l-epigallocatechin and d,l-epicallocatechingallate occurred in considerably smaller quantities than in the leaves, whereas l-epicatechingallate was completely lacking. There were even fewer free catechins in the roots. In the chromatograms of the roots, in addition to considerable residues (products of catechin condensation) only weak spots of l-epicatechin, d,l-gallocatechin, and l-epigallocatechin were disclosed. Thus, in accordance with data obtained earlier for sprouts [12], the roots of the tea plant are, apparently, not capable of synthesizing gallic forms of catechins, and this ability is considerably decreased in the bark. If we assume that no catechins are formed in the bark or roots of mature tea plants, but are translocated to these tissues from the leaves, then it is apparent that only simple catechins (nongallic) are capable of this translocation.

The specific activity of catechins in young shoots and mature leaves of intact plants was determined after their elution from the chromatograms (see methods). The data obtained are given in Table 5.

From Table 5 we can see that the catechins in the young three-leaved shoots had on the average, a radioactivity 26 times greater than the mature (fifth-sixth) leaves. Furthermore, the capacity for catechin synthesis in the young shoots was ten times as great as it was in the mature leaves.

TABLE 5

Specific Activity of Catechins in Young Three-Leaved Shoots and Mature (Fifth-Sixth) Leaves (in counts/ min/ mg)

Catechins	Young shoots	Mature leaves	Ratio of specific activity: Young shoots / Mature leaves
l-epigallocatechin	9840	243	40.5
d,l-gallocatechin	16570	700	23.7
l-epicatechin+d,l-catechin	18000	750	24.0
l-epigallocatechingallate +l-gallocatechin-gallate	9570	327	29.3
l-epicatechingallate	12670	944	13.5

In addition to this, individual catechins did not become labeled uniformly. The greatest specific activity was exhibited by the catechins with the ortho-placed oxy group (l-epicatechin, d,l-catechin, and l-epicatechingallate) in the young shoots as well as in the mature leaves, whereas d,l-gallocatechin existed only in small quantities in the tea leaves. The specific activity of the basic component (up to 60% of the total catechins) of tea catechins — l-epigallocatechingallate — is approximately ½ as great as the activity of l-epicatechin. Such a result agrees to a certain degree with data obtained earlier from tea sprouts [12] concerning the appearance of l-epicatechin as the most simple transformable catechin, from whose molecules remaining representatives of this group are later synthesized.

Even though the photosynthetic activity of mature leaves was lower in comparison with that in the young shoots, the rate was quite adequate judging from the specific radioactivity of the sugars (Table 6).

The radioactivity of the sugars in the mature leaves was on the average only one-half as great as it was in the young shoots; this can be explained to some extent by the translocation of photosynthates out of them. Therefore, the photosynthetic activity of mature tea leaves is apparently directed primarily to the synthesis of the basic building material — the sugars, which are translocated from there throughout the plant. Such a conclusion agrees with previously reported data [9, 20] that as the growth of the tea leaves increases, the amount of tannins in them decreases, and the sugar content increases.

It can be assumed that a considerable portion of the sugars formed as the result of photosynthesis are used in the synthesis of tannins in the young shoots. The question is whether this synthesis occurs as in intermediate formation of inositol [9, 21] or shikimol acid [22, 23]; this was not investigated in this study.

The sugars synthesized in the mature leaves are, apparently, used primarily as a source of energy for respiration and growth of the roots and new shoots. Thus, as the tea leaves increase in size they gradually lose more and more of their ability to synthesize catechins.

TABLE 6

Specific Activity of Sugars in Young Three-Leaved Shoots and Mature (Fifth-Sixth) Leaves (in counts/min/mg)

Sugars	Young shoots	Mature leaves	Ratio of specific activity: Young shoots / Mature leaves
Raffinose	80 000	59 000	1.34
Sucrose	71 000	33 800	2.10
Glucose	96 000	70 800	1.36
Fructose	97 300	30 300	3.21

Experiments in progress at the present time with shorter exposures will help answer the question concerning rate and sequence of the photosynthetic formation of catechins.

After the present investigation was completed, an article appeared by a group of German investigators [24] in which the authors reported that after infiltrating the tea leaves with inositol containing labeled C^{14}, or else immersing isolated leaves in a solution of labeled inositol, they were unable to detect the formation of labeled catechins. On this basis they concluded that inositol is not used in the tea plant as an intermediate product in catechin synthesis. Since they used mature leaves in which growth had ceased (the authors report that only in the experiment where the freshly cut leaves were immersed in a solution of C^{14}-meso-inositol – were one out of three leaves "still growing"), in light of data presented above the results obtained by the German investigators cannot be considered conclusive. It is quite possible that mature leaves which had generally lost their ability to synthesize catechins to a considerable degree would not be able to synthesize them from inositol either. Experiments introducing labeled inositol should be performed on young, rapidly growing leaves which synthesize catechins vigorously.

Various polyphenol compounds and their condensation products – tannins – are widely distributed in the plant world. However, the physiological role of these compounds is not well known. The function of polyphenols as carriers of hydrogen atoms in the process of respiration, as protective factors during injury or infectious diseases, or their role in various secondary reactions of oxidation [9] does not require an accumulation of significant quantities of tannins; this has frequently seen observed in very diverse plants. The vigorous synthesis of catechins in young rapidly growing parts of tea plants, shown in this investigation, indicates a more active role of polyphenols in metabolic processes. It is difficult to visualize that the metabolism of young tissues with the most active growth processes should be directed to the synthesis and accumulation of compounds which in the future would only infrequently play an active role.

It is known that microorganisms are capable of completely uncoupling the phenol rings via intermediate synthesis of cis-cis-muconic acid and β-ketoadipic acid [25-27]. It has recently been shown that there also exists an anaerobic respiratory system in the liver of mammals which brings about the complete oxidation of l-tyrosine, l-phenylalanine, rutin, and flavin [28-30]. Therefore, microorganisms and mammals can utilize polyphenol compounds, and recover the energy stored in them. However, the ability to uncouple the phenol ring had not been observed in higher plants until the present. Future experiments using labeled atoms will undoubtedly help to establish the fate of polyphenols in the tea plant more accurately.

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SUMMARY

1. Ninety-two hours after photosynthetic introduction of $C^{14}O_2$ into tea plants most of the radioactivity (up to 84%) was centered in the young shoots and mature leaves. In addition, the highest rate of C^{14} incorporation was observed in the young, three-leaved shoots.

2. Since the synthesis of catechins from $C^{14}O_2$ occurred at the same rate in plants with exised roots as in intact plants, apparently the formation of tannins (catechins) in the leaves and young shoots of the tea plant

is not directly related to the activity of the roots.

3. A study of the distribution of radioactivity of different groups of compounds revealed that whereas the source of radioactivity in the mature leaves was almost exclusively sugar, in the young shoots about 25% of the total radioactivity of an alcohol extract was due to catechins.

4. Catechins in the bark, wood, and roots had practically no radioactivity when compared, for example, with their radioactivity in mature leaves.

5. A comparison of the specific activity of individual catechins and sugars in young three-leaved shoots and mature leaves revealed that although the activity of the sugars in the young shoots was, on an average, only twice as great as it was in the mature leaves, the activity of the catechins in the young shoots was, on an average, 26 times greater than the activity in the mature leaves. Therefore, there is apparently a specialization in the transformation of photosynthates in leaves at various levels in the tea plant. In the mature leaves which had ceased growing, sugar was the principal product of photosynthesis; this was used primarily as a source of energy in the process of respiration, and the growth of roots and new shoots. In the young rapidly growing leaves, however, photosynthesis led to the formation of catechins as well as sugars.

6. Since almost no catechins were formed during photosynthesis in the mature excised leaves, while excised shoots retained this ability completely, one can conclude that the young rapidly growing shoots are the main sites of catechin synthesis in the tea plant.

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PASSAGE OF WINTER CEREALS THROUGH THE VERNALIZATION STAGE UNDER FIELD CONDITIONS

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The literature of the biological sciences contains much information concerning the conditions of vernalization and its duration in winter cereals. However, practically all this information has been obtained under laboratory conditions which are far removed from the natural conditions in the field.

As a rule, in these experiments the duration of the vernalization stage in one or another of the winter varieties was determined by the vernalization of germinating seeds during a various number of days; following this they were planted in a greenhouse or else outdoors in the soil in the spring. A study of the passage of the vernalization stage in winter varieties at field conditions is important since it will give a more successful guide to the nature of winter hardiness in plants.

There is practically no information in the literature concerning the rate of vernalization and its completion in winter cereals at field conditions. Because of this we started an experiment in 1950 in order to study the progress of the vernalization stage in winter crop plants under natural field conditions with the object of ascertaining the completion of the vernalization stage and the rate of its passage.

The studies were begun at the Moscow Selection and Breeding Station of the K. A. Timiriazev Agricultural Academy in 1950-1951, and were continued at the Institute of Genetics of the USSR Academy of Sciences (1952, 1955).

Fourteen varieties of wheat were used as experimental material: Liutestsens 329, Kooperatorka, Moskovskaia 2453, Diurabl', Liutestsens 116, Dmitroskaia (Al'borubrum), Wheat-couch grass hybrid 599, Hybrid No. 1, branching hybrid (F_6, F_7), winter wheat selected from summer Mil'turum 321 near Moscow, winter wheat selected from summer Mil'turum 321 near Omsk, winter wheat selected from summer Mil'turum 321 at Odessa, Bankuti 1201, partially hard Apulikum (Zarda), and two varieties of winter rye — Viatka moskovskaia and Timiriazevskaia.

In 1952, together with plantings in the field, sowing were also made in containers which were dug into the open soil. In view of the fact that we observed no noticeable variations in the passage of the vernalization stage between plants sown in the field of those in containers, in this description of the material we shall not take time to point out the data concerning passage of the vernalization stage in plants in the containers.

The method of determining the completion of the vernalization stage under natural conditions was as follows. Beginning October 1 plants were dug out of the field every five days and transplanted into medium sized containers. The plants were taken with a lump of soil in order not to damage the root system seriously. The containers with the plants were then transferred to the greenhouse and placed under natural light. When the plant samples were taken after the soil had frozen, plants with a clump of soil were brought into a cold place for 1-2 days, and then after the soil had thawed they were transplanted into pots. Five days after the sample had been taken, when the transplanted plants had begun to grow, the pots with the plants were transferred to continuous illumination.

The source of supplementary illumination was primarily 500 watt incandescent bulbs, and sometimes luminescent 30 watt DC bulbs. The temperature in the greenhouse ranged from 16 to 20°.

Plant samples were brought in from the field principally during two months (October, November), and in some cases even later.

It is well known that the differentation of the apical meristem and the subsequent appearance of heads in winter wheat can only occur after the vernalization stage is completed. Formation of the embryonic head indicates that the vernalization stage in the specific plant has been completed. One can more accurately determine whether the vernalization stage is completed by the differentation of the apical meristem than by the appearance of the spike since the process of differentation itself and the growth of the spike occur at different rates, depending on environmental factors. By placing the plants under continuous illumination and gradually increasing the temperature (from 16 to 20°), we created a favorable environment in this way for the light stage and the formation of reproductive organs. As a result, the plants in which the vernalization stage was completed at the time they were taken from the field as samples were able to begin the formation of reproductive organs. The plants in which the vernalization stage was not completed were unable to form reproductive organs (within the usual time period) and develop spikes.

TABLE 1

The Number of Days from the Time the Plants Were Placed Under Continuous Illumination in the Greenhouse Until the Differentation of the Apical Meristem Depending on the Planting Period and the Time They Were Brought in from the Field

(1954 Experiment)*

Date plants brought in from field	Planting dates: Aug. 2	Aug. 12	Aug. 22	Sept. 7	Sept. 22	Oct. 7	Date plants brought in from field	Planting dates: Aug. 2	Aug. 12	Aug. 22	Sept. 7	Sept. 22	Oct. 7
	Liutestsens 329							Moskovskaia 2453					
Oct. 20	29	34	—	—	—	—	Oct. 20	—	—	—	—	—	—
Oct. 25	21	23	—	—	—	—	Oct. 25	17	17	—	—	—	—
Oct. 30	7	10	—	—	—	—	Oct. 30	7	9	21	44	—	—
Nov. 5	9	9	14	17	37	—	Nov. 5	8	8	16	24	32	—
Nov. 10	7	7	11	15	21	—	Nov. 10	8	8	12	17	30	—
Nov. 15	8	8	10	12	16	30	Nov. 15	6	8	9	14	23	—
Nov. 20	8	8	10	13	17	24	Nov. 20	7	8	10	13	18	25
	Kooperatorka							Timiriazev Rye					
Oct. 20	14	14	—	—	—	—	Oct. 20	9	9	—	—	—	—
Oct. 25	7	9	18	24	—	—	Oct. 25	9	9	23	—	—	—
Oct. 30	8	8	8	17	25	—	Oct. 30	9	9	12	25	—	—
Nov. 5	7	7	7	10	22	—	Nov. 5	8	8	11	15	24	—
Nov. 10	7	7	8	10	15	19	Nov. 10	8	8	10	11	12	—
Nov. 15	7	7	8	10	12	16	Nov. 15	8	8	10	11	12	—
Nov. 20	7	7	7	10	11	16	Nov. 20	8	8	10	11	12	—

*For brevity the data conceming plants with spikes is not included.

We determined the termination of the vernalization stage on the basis of observations on the development of the apical meristem and the time spike formation began. For example, in the winter wheat Kooperatorka which was planted August 12, 1953 and transferred to the greenhouse on October 20, differentation of the apical meristem began after 9 days of continuous illumination, whereas spike formation was recorded on December 12. This indicated clearly that the vernalization stage in these plants had been completed by October 20.

Kooperatorka plants from this sowing period which were taken up from the field on October 1 did not develop spikes after 30 days of continuous illumination; this indicated that the vernalization stage was incomplete by October 1. However, during the course of the investigation we encountered several difficulties because

plants in which the vernalization stage was almost completed were able to complete this stage even under unfavorable conditions – in the greenhouse (at increased temperatures). Therefore, in order to be more accurate we recorded the number of days at continuous illumination which had elapsed from the time the plants were placed under continuous illumination until the differentation of the apical meristem in a given variety from a specific planting period at various periods of transfer from the field into the greenhouse.

By comparing the results obtained we came to a conclusion concerning the time the vernalization stage ceased; this can be seen from the data in Table 1.

From an analysis of the data in Table 1, one can easily establish the time the vernalization stage was complete in one or another variety of wheat or rye. For example, in winter wheat plants of Liutestsens 329 planted on August 12 the embryonic spikes began to appear within 34 days when the plants were transplanted from the field to the greenhouse on October 20, but when they were transplanted on October 25 the spikes appeared after 23 days, those transplanted October 30 after 10 days, and in those transplanted November 5 after 9 days, etc. This is the basis for considering the vernalization stage of winter wheat plants Liutestsens 329 completed by October 30. A similar time period was also established for the termination of the vernalization stage at field conditions in other winter plants.

Additional data which we used to determine the termination of the vernalization stage in winter plants consisted of the time required for the onset of complete spike formation, this occurred in the greenhouse about 30-40 days after differentiation of the apical meristem.

Usually the winter plants formed spikes during the second half of December and in January. In addition, it should be noted that the data observed for the spike formation agreed completely with data observed for differentiation of the apical meristem.

By using the method described above we determined the time necessary for the vernalization stage to terminate in winter cereals at field conditions.

In view of the fact that the data from the previous years was similar to the data of the last three years, Table 2 contains results of experiments concerning the termination of the vernalization stage during 1952-1954.

The data in Table 2 show that in the environs of Moscow the vernalization stage of winter cereals terminated the end of October and in November. Small variations, on the order of 5-12 days, were observed during specific years. Plants sown in August completed the vernalization stage earlier than those sown in September. Most of the varieties planted at optimum periods completed the vernalization stage by the end of October in 1953, but not until the first ten-day period of November in 1954. Plants from the September sowing terminated the vernalization stage the middle of November during the three years. After the October sowing (October 7) in 1953, there was no germination recorded that fall, nevertheless even during such a late sowing the vernalization stage was terminated in November (the vernalization was completed in the seeds).

In 1953 there was a frost during the night of October 29-30 (5.8°). As a result the soil froze 3-4 cm; on November 14 and December 7, because of thawing, we made fresh sowings of winter plants.

The data in Table 2 show that the plants from these periods had completed the vernalization stage only toward the end of spring (the sprouts from these plantings appeared in the spring, in April) as well as in the plantings on October 7. It may be assumed that after sowing at these very late periods the seeds began to pass through the vernalization stage in the germinating condition, but were unable to complete it during the winter because of low temperatures. They completed the vernalization stage no sooner than by the end of spring. These data show that only when the seeds were sown very late, which does not occur in agricultural practice did the winter wheats enter the winter rest period before the vernalization stage was completed.

In 1953 the plants from the August sowing completed the vernalization stage before the first frost (October 30), but in 1954 not only the plants from the August sowing, but also those from the September sowing, completed the vernalization stage before the first severe frosts (November 20).

According to Tumanov [1] the second stage of hardening occurs during below-zero temperatures (– 2° to – 5°) in plants. From this it appears that the second stage of hardening in winter plants from optimal sowing periods occurs after the vernalization stage is completed.

From the data in Table 2 it also appears that the more winter resistant varieties of wheat completed the

TABLE 2

Termination of the Vernalization Stage in Winter Wheats and Rye at Field Conditions in Different Varieties and at Different Sowing Dates

Date of sowing	Completion of the vernalization stage in 1952	in 1953	in 1954
	Liutestsens 329		
Aug. 2	—	Oct. 25	Oct. 30
Aug. 12	Nov. 10	Oct. 25	Oct. 30
Aug. 22	Nov. 12	Oct. 30	Nov. 10
Sept. 7	Nov. 18	Nov. 10	Nov. 15
Sept. 22	Nov. 20	Nov. 15	Nov. 20
Oct. 7	Nov. 20	Nov. 20	Nov. 25
Dec. 7	—	May 20-25, 1954	
	Kooperatorka		
Aug. 2	—	Oct. 17	Oct. 25
Aug. 12	—	Oct. 20	Oct. 25
Aug. 22	—	Oct. 20	Oct. 30
Sept. 7	—	Oct. 30	Oct. 5
Sept. 22	—	Nov. 10	Nov. 10
Oct. 7	—	Nov. 15	Nov. 10
Dec. 7	—	May 12-15, 1954	
	Moskovskaia 2453		
Aug. 2	Nov. 10	Oct. 30	Oct. 30
Aug. 12	Nov. 10	Oct. 30	Oct. 30
Aug. 22	Nov. 12	Oct. 30	Nov. 10
Sept. 7	Nov. 18	Nov. 10	Nov. 15
Sept. 22	Nov. 20	Nov. 15	Nov. 20
Oct. 7	—	Nov. 20	Nov. 25
Nov. 14	—	May 10, 1954	
Dec. 7	—	May 25-28, 1954	
	Diurabl'		
Aug. 2	—	Oct. 30	Oct. 30
Aug. 12	—	Oct. 28-30	Oct. 30
Aug. 22	—	Oct. 30	Nov. 10
Sept. 7	—	Nov. 15	Nov. 15
Sept. 22	—	Nov. 15	Nov. 20

Date of sowing	Completion of the vernalization stage in 1952	in 1953	in 1954
	Liutestsens 116		
Aug. 2	—	Oct. 30	Oct. 30
Aug. 12	—	Oct. 30	Oct. 30
Aug. 22	—	Oct. 30	Nov. 5
Sept. 7	—	Nov. 15	Nov. 15
Sept. 22	—	—	Nov. 20
	Dmitrovskaia (Al'borubrum)		
Aug. 22	—	Nov. 5	Nov. 10
Sept. 7	—	Nov. 15	Nov. 20
Sept. 22	—	—	—
	Wheat-Couch Grass Hybrid 599		
Aug. 2	Nov. 10	—	Oct. 30
Aug. 12	Nov. 10	Oct. 25	Oct. 30
Aug. 22	Nov. 14	Oct. 30	Nov. 10
Sept. 7	Nov. 18	Nov. 10	—
Sept. 22	Nov. 20	—	Nov. 20
	Bankuti 1201		
Aug. 22	—	—	Oct. 15
	Apulikum		
Aug. 22	—	—	Oct. 5-10
	Timiriazev Rye		
Aug. 2	Oct. 30	Oct. 20	Oct. 20
Aug. 12	Nov. 3	Oct. 20	Oct. 20
Aug. 22	Nov. 10	Oct. 28-30	Oct. 30
Sept. 7	Nov. 12	Nov. 7	Nov. 5
Sept. 22	Nov. 18	Nov. 10	Nov. 10
Nov. 14	—	May 10-15, 1954	
Dec. 7	—	May 3-10, 1954	
	—		
	Viatka Moskovskaia		
Aug. 22	—	Oct. 30	Oct. 30
Sept. 22	—	Nov. 10	Nov. 10

vernalization stage later than those which are mildly resistant. The first to complete the vernalization stage in 1954 were the semi-winter wheat Apulika (October 5-10) and the winter wheat Vengerskaia from the selection Bankut 1201. Of the winter wheats cultivated in the Soviet Union, the one which completed the vernalization stage earlier than the others in our experiments was Kooperatorka. The other winter wheats completed this stage somewhat later than Kooperatorka. There were no significant variations observed in our experiments in the time the vernalization stage was completed among the winter resistant winter wheats.

The data in Table 2 show that in winter rye the vernalization stage was completed somewhat earlier (5-10 days) than in the winter wheats.

In 1953 at our request some work was done on determining when the vernalization stage was completed in the winter wheat Moskovskaia 2453 and in winter rye Timiriazevskaia by a student at the G. Fedorov Leningrad Agricultural Institute. This work revealed that in the environs of the Pushkin Mt. near Leningrad the vernalization stage in winter wheat Moskovskaia 2453 and in winter rye Timiriazevskaia was also completed in November.

By comparing the data at which the vernalization stage is completed at field conditions with the weather conditions one can approach a solution to the problem of the rate at which the vernalization stage progresses in the winter plants under field conditions.

In 1952 experiments concerning the completion of the vernalization stage were performed in the vicinity of (100 m) of the weather station at the Moscow K. A. Timiriazev Agricultural Academy. An analysis of the temperature data* obtained at this station shows that in 1952 the first drop in temperature (to 0°, + 2°) to that optimum for the progress of vernalization in winter crops occurred on September 16 and 17 (+ 1.5 to 1.9°), following that the minimum temperatures during the third ten-day period in September dropped within the range from + 2.9 to + 11.7°. The average temperature during the third ten-day period of September as well as during the second ten-day period was considerably higher than the optimum temperature for the progress of the vernalization stage in winter crops, and consequently averaged + 9.2 and + 9.3°. The average temperature during the second ten-day period of October was close to the optimum (+ 4.9°). Only during the third ten-day period in October did the average temperature within the limits favorable for progress of the vernalization stage become established (average temperature during third ten-day period of October was + 1.1°).

During the first ten-day period (October 4) there was a frost and the temperature dropped to − 3.8 to − 6.5°. The average temperature during the first ten-day period in November was + 1.8°. During the second ten-day period the average temperature was, as a rule, below 0 (− 1.7°). This temperature was below the optimum established as necessary for progress of vernalization under laboratory conditions.

From the data in Table 2 it is evident that the winter rye from the August sowing completed the vernalization stage between October 30 and November 10 in 1952. An analysis of the temperature data shows that the plants from these sowings were exposed to average daily temperatures which were optimal for the progress of the vernalization stage during the third ten-day period in October (+ 1.1°) and during the beginning of November (+ 1.8°), i.e., for 10-20 days. If we consider the minimum temperatures to which the plants were exposed for only a short time during the day, then the optimum temperature (0, + 2°) or that close to the optimum began on October 1.

From October 1 to November 1, when the vernalization stage of rye was completed, 30 days had elapsed. However, it is necessary to take into account the fact that during the period from October 1 to November 20 the optimum temperatures, or those close to the optimum for the vernalization process, had occurred only during the night. From this we see that of the 20 days in October only about 10 of them were actually favorable for the vernalization process.

It is generally known that the vernalization stage of rye in germinated seeds is completed after 40 days at optimum temperatures (0°, + 2°) (Razumov [2]). Vernalization occurs considerably slower at other temperatures. According to Razumov [2], at − 4° the vernalization stage was completed after 70 days. During daily variations of high and low temperatures the vernalization process was also considerably slower.

From the data in Table 2 it is apparent that by November 10-22 the vernalization stage in winter resistant wheats such as Liutestsens 329, Moskovskaia 2453, and wheat-couch grass hybrid 599 was already completed. From data in the literature it is known that these wheats have a vernalization stage which exists for about 50 days. A study of the weather data reveals that these wheat plants were capable of passing through the vernalization stage at more or less optimal daily temperatures during only 20 days.

Average temperature below 10° played a role during the third ten-day period of September. Minimum temperatures which are close to the optimum temperatures required for the progress of vernalization were established during the first ten-day period in October. This means that plants were able to proceed through the vernalization stage for only a few hours each day at optimum temperature. At best, of the 20 days (first and second ten-day period in October) only 10 days had minimum temperatures which were close to optimal

* Air temperature.

for vernalization, since apparently the temperature was close to optimal for vernalization for somewhat less than 12 hours a day.

Therefore, during the progress of the vernalization stage there were about 30 days with temperature close to the optimal. From this we conclude that winter rye and wheat at field conditions pass through the vernalization stage at the same rate as they do at laboratory conditions as germinated seeds at optimum conditions (0°, + 2°). Winter rye and winter wheat begins the vernalization stage at considerably higher temperatures as compared with the optimum temperatures required for germinated seeds to become vernalized at laboratory conditions.

The total length of time required for the vernalization of green plants in the fall was somewhat shorter than that for the vernalization of germinated seeds of the same varieties of plants. We believe that this can partially be explained by the short autumn days which contribute to the rapid progress of vernalization.

We will pause briefly to analyze the temperature data for 1953 in relation to the progress of vernalization in winter plants.

During the first, second, and third ten-day periods in September the average temperature was about + 10°. During the first and second ten-day period of October the averate temperature was also close to this (+ 7°, + 8°). And only during the third ten-day period was the average temperature + 2°, i.e., optimal for vernalization.

Minimum temperatures which were comparatively lose to the optimal for the progress of vernalization were of significance at the end of the first and second ten-day period of October. In November, temperatures below 0° became established.

From the data in Table 2 it appears that winter rye Timiriazevskaia and winter wheat Kooperatorka from the August sowings, also the optimum ones, completed the vernalization period by October 20.

Temperatures which are considered optimal for the progress of vernalization (0°, + 2°) occurred only during the third ten-day period of October. From this it appears that in the given plants the vernalization stage occurred and was completed at temperatures above 0°, + 2°. Frost resistant winter wheats of these sowings completed the vernalization stage by October 30. While they were passing through the vernalization stage there were only ten days with optimal temperatures throughout the day. This is somewhat the basis for concluding that at field conditions probably the optimal temperature relations for the occurrence of vernalization differ to some degree from the optimum temperature relations established for the occurrence of vernalization under laboratory conditions.

A comparison of temperature data with the progress of vernalization of winter plants in 1953 reveals that at natural conditions the vernalization stage begins at comparatively higher temperatures and progresses no slower than it does during vernalization of germinated seeds at optimal temperatures (0°, + 2°) under laboratory conditions.

The autumn of 1954 was warm, the average temperature was below 10° only during the third ten-day period of September. Minimum temperatures, close to those optimum for the progress of vernalization, occurred during October and the first half of November. The average temperature during the first and second ten-day period of October and the first ten-day period of November did not drop below + 5°.

In spite of the relatively warm fall, the vernalization of winter plants in 1954 was completed with only a slight delay (average of about 5 days) as compared with that in 1953. Winter resistant wheat plants from the August sowing in 1954 completed the vernalization stage by November 10.

An analysis of meteorological data shows that until November 10th there were only several days (18th, 19th, and 20th of October) when the average daily temperature was within the limits of 0°, + 2°. During the remaining days of the period in which vernalization of winter plants occurred, the average daily temperature was higher than +2°. This indicates that the vernalization stage of winter plants in the field generally occurs at higher temperatures than those considered optimal for vernalization of germinating seeds under laboratory conditions. The total duration of the vernalization stage of green plants in the fall was somewhat less than that of germinating seeds of the given varieties during their vernalization at natural conditions (at 0°, + 2°). This latter, in our opinion, can be partially explained by the existence of a short day together with other factors favorable for vernalization, especially low temperatures, which favor a more rapid progress of the vernalization stage.

If we compare the temperatures in the fall of 1953 with those in the fall of 1954, it is apparent that the fall of 1954 was warmer than the fall of 1953. This undoubtedly affected the vernalization of the winter plants. From the data in Table 2 it is apparent that practically all the varieties from all the sowing dates completed the vernalization stage in 1954 somewhat later than in 1953. However, in 1953 as well as in 1954 the vernalization stage in the winter plants sown at an optimal date was completed by the onset of stable winter weather.

Hence, under natural conditions (of Moscow) the vernalization stage of winter cereals was completed in the end of October and in November. During natural conditions the vernalization stage of winter plants progressed no slower than under laboratory conditions at optimum temperatures (0°, +2°). As our three-year data show, at optimal sowing dates (August 15-25) the vernalization stage of winter wheats and rye is completed by the time of the first severe frosts at the end of October and the first ten-day period of November.

In fact, vernalization of winter plants is, as a rule, completed by the onset of stable winter weather. And it is also an established rule, that within a given period, in spite of the completion of the vernalization stage, the apical meristem cannot differentiate because of unfavorable conditions for the light stage and the formation of reproductive organs. Therefore, the termination of vernalization at these conditions cannot generally have a negative effect on the winter resistance of plants. It is known that winter plants exhibit the greatest winter resistance in the beginning of winter.

SUMMARY

The vernalization stage of winter wheats and rye planted in the field in August and early September in the environs of Moscow occurred principally at temperatures somewhat higher than optimal (0°, + 2°) which have been established as those required for the vernalization of germinated seeds. The duration of the vernalization stage of green plants was somewhat shorter than it was in the same varieties of germinating seeds under experimental conditions.

In the environs of Moscow the vernalization stage in winter wheats and rye terminated in late October and November depending on the variety, date of sowing, and weather conditions.

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FORMATION AND TRANSFORMATION OF ORGANIC ACIDS IN TEA SPROUTS

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The organic acids of tea have not been extensively investigated, although a number of papers have been devoted to them [1-9]. Even so, it is known that organic acids play in important part in plant metabolism. Investigation of the acid exchange in the tea plant is interesting particularly because it may give a clue to the nature of the intermediate respiratory products in plants capable of a copious formation of phenolic substances, the content of which reaches 30-35% in a tea leaf.

The purpose of the present work was to elucidate the formation and transformation of organic acids in the process of germination of tea plant seeds and in the development of young tea seedlings.

A number of investigations have been devoted to the transformation of organic acids in the course of development of various plants from seeds to the mature state. In particular, the data of Soldatenkov with collaborators [10] show that a sharp increase in organic acid content takes place during germination. Here, as these authors point out, mostly 2- and 3-carbon acids are formed. The active formation of organic acids in seeds during germination once more confirms the important physiological significance of organic acids in the life of plants.

METHODS

In our work, we used seeds collected from the plantation of the Institute of Tea and Subtropical Crops. Seeds were germinated in the sand. Analyses were made of: 1) dormant tea seed; 2) 40-day-old tea seedlings; 3) cotyledons of germinated seeds (40 days after germination); 4) roots of 60-day-old seedlings; 5) stems with buds of 60-day-old seedlings; 6) first true leaf of 100-day-old tea plants.

The fresh material was gound with solid carbon dioxide, after which 25% H_2SO_4 was added to the material, using 0.5 ml of acid per gram of material. The material was left in acid for 15 to 17 hours and was then mixed with Na_2SO_4 (20 grams per gram wet weight). From the material thus dehydrated, organic acids were extracted in a Soxhlet apparatus with dry sulfuric ether freed of peroxide. Extraction was continued for thirty hours. The extract, thus obtained, was evaporated to dryness, and the dry residue was dissolved in a small amount of distilled water.

Organic acids were determined by the method of descending paper chromatography [11]. As a solvent, we used a mixture of n-butanol: formic acid: water (180: 30: to saturation). The extracts obtained were placed on strips of chromatographic paper No. 2 (Volodarskii Factory). At the same time, solutions of organic acids were applied to the paper as controls. After 15 hours, the chromatogram was dried at room temperature and was then sprayed with a 0.04% solution of bromphenol blue for identification of acids.

From drawings of chromatograms given below, we see that citric acid and traces of malic and oxalic acids are found in the dormant tea seeds. Citric acid is predominant in quantity (Fig. 1). This fact is interesting since, according to some authors [10], citric acid may be regarded as the reserve form of sugar, physiologically speaking, since it retains the carbon and a large part of the energy of the sugar molecule, and may be used for respiration, as well as for the carbon skeleton for the synthesis of a number of compounds.

Further consideration of the chromatogram (see Fig. 1) shows that an accumulation of considerable amounts

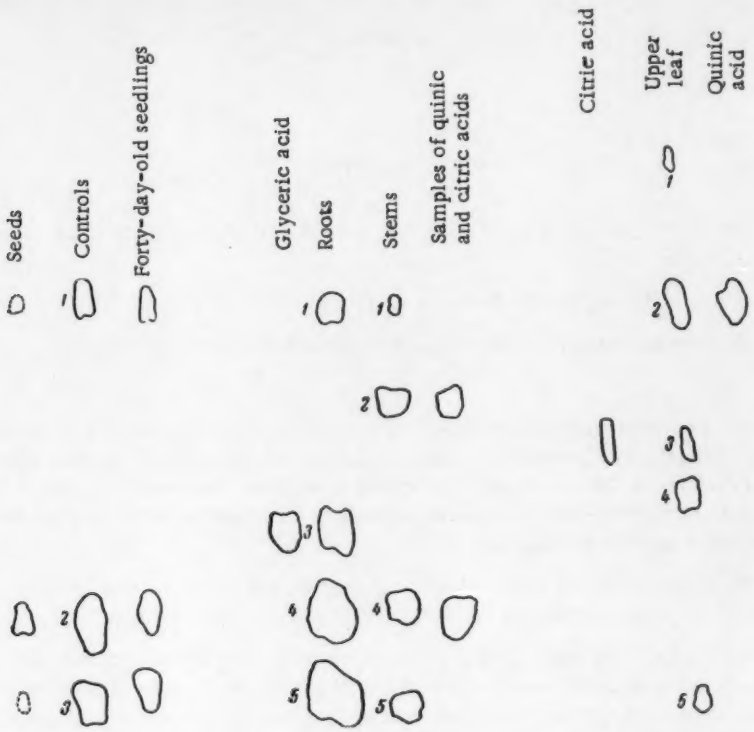

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Chromatogram of organic acids in seeds and forty-day-old seedlings of tea. 1) Oxalic; 2) citric; 3) malic.

Fig. 2. Chromatogram of organic acids in the roots and stems of sixty-day-old tea plants. 1) Oxalic; 2) quinic; 3) acid "X"; 4) citric; 5) malic.

Fig. 3. Chromatogram of organic acids of tea leaf. 1) Oxalic; 2) quinic; 3) citric; 4) malic; 5) fumaric.

of malic acid takes place in 40-day-old tea seedlings. As is known, malic acid is one of the important links in oxidative transformation during respiration. Since respiration energy in germinating seeds is higher than in dormant seeds, it is possible that the accumulation of malic acid in 40-day-tea sprouts takes place as a result of a sharp increase in respiration during seed germination. In addition, amination of malic acid forms asparagine, which is found in large amounts in germinating seeds [12]. Asparagine may play the part of a reserve protein of seeds and of a constitutional protein of young plants. In itself carboxyl, malic acid may also transport into leaves the carbon dioxide absorbed from the soil by roots, as Kursanov has shown in a number of plants [13].

In connection with the material presented above, it seemed interesting to determine the location of the synthesis of malic acid in the period of seed germination. For clarification of this question, we analyzed cotyledons of 40-day-old seedlings. The organic acid content in the cotyledons of germinated seeds is analogous to their content in 40-day-old tea seedlings. These data permit one to suppose that the energetic synthesis of malic acid begins during germination of tea seeds, particularly in the cotyledons.

It turned out that in the latter stages of development of young plants, after appearance of sprouts (60th day after germination), a sharp difference is found in the composition of organic acids in different parts of the seedlings. The roots of 60-day-old seedlings are distinguished from stems of the same age by both the quantitative and qualitative composition of organic acids. Often it was found that the roots of 60-day-old seedlings, as compared with stems of the same age, contained significantly greater amounts of malic, citric, and oxalic acids. This

TABLE

Content of Citric and Malic Acids in Dormant Tea Seeds and in Seedlings

Experimental material	Citric acid in mg/g dry wt.	Malic acid in mg/g dry wt.
Seeds	3.5	1.0
40-day-old seedlings	4.5	8.5
Roots of 60-day-old seedlings	6.0	12.5
Stems of 60-day-old seedlings	3.1	5.4

confirms new investigations of Kursanov and his collaborators [14], obtained with a different plant material, which show that it is the root system that supplies the plant with many organic substances.

In addition, at this stage of development in the roots of 60-day-old tea seedlings another organic acid, as yet not identified by us, was found; according to its location on the chromatogram this acid corresponds to glyceric acid (Fig. 2). It was also found chromatographically that a synthesis of quinic acid is initiated in the stems of 60-day-old seedlings. According to the proposal of Blagoveshchenskii [15], quinic acid takes a direct part in the synthesis of gallic acid. Experimental data of Kursanov [16] showed that infiltration of quinic acid in tea leaves contributes to the synthesis of polyphenols with serial oxy-groups. All this permits one to suppose that the formation of quinic acids in the tea seedling stem should be considered to be connected with the synthesis of tannic substances, which is already initiated in this stage.

This proposal is confirmed also by the data obtained in analysis of the first true leaves of 100-day-old tea plants. From these data, it can be seen that quinic acid is quantitatively predominant over other acids in the first true leaves of 100-day-old seedlings (Fig. 3). Since the first true leaves of the 100-day-old plant already contain significant amounts of tannic substances, one may assume that the intensified synthesis of quinic acid is connected with the rapid formation of tannic substance. It should be noted also that in the first true leaves of the 100-day-old tea plants small amounts of fumaric acid were found.

Along with chromatographic analysis, we have made a quantitative determination of citric and malic acids in dormant tea seeds, in 40-day-old seedlings and, in the stems and roots of 60-day-old seedlings of the tea plant.

Citric acid was determined by the pentabromacetone method, as modified by Pucher. The method is based on the oxidation of citric acid in the presence of brominating agents in pentabromacetone. Citric acid was then determined colorimetrically by the color which is developed after interaction of pentabromacetone with Na_2O. The amount of citric acid was calculated from a calibrated curve [17].

Malic acid was determined by the dinitrophenylhydrazine method developed by Pucher. The 2,4-dinitrophenylhydrazone of glyoxal, which is formed by oxidation of malic acid, gives a color reaction with NaOH, which is measured colormetrically with the aid of a photoelectric colorimeter. The amount of malic acid is also calculated from a calibrated curve [18].

The data obtained are summarized in the table.

The data of the table, showing quantitative determinations of organic acids, completely confirms the dynamics of citric and malic acids during germination of tea seeds which we established with the aid of chromatographic methods.

SUMMARY

The results obtained show that dormant tea seeds contain citric acid and traces of malic and oxalic acid.

As the seeds germinate (40 days after planting), there is a sharp increase (8 times) of the malic acid content in the seedlings.

In the later stages of development of the tea seedling (60-day-old seedling), the roots begin to synthesize an organic acid, which in its R_f value and its position on the chromatogram corresponds to glyceric acid.

Sixty-day-old stems of tea seedlings contained half as much citric and malic acids as compared with roots of the same age, and in addition, according to our preliminary data, they begin to synthesize quinic acid.

In 100-day-old tea seedlings, small amounts of fumaric acid were found.

SHORT PAPERS

THE DORMANT STATE AND PHYSICOCHEMICAL PROPERTIES OF THE CELLS IN ROOTS AND BUDS OF RED CLOVER PLANTS

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Characteristic properties of the dormant state are a decrease in the rate of physiological properties, particularly the cessation of visible growth in the plant as a whole and in the individual dormant organs, and a decrease in the metabolic rate [1, 2].

The papers of Genkel and Oknina [3, 4] show that the protoplasm breaks from the cell wall and plasmodesmata disappear in the cells of dormant plants. The plants are more frost-resistant in the dormant state [5].

The dormant state in red clover plants were studied by Kolomiets [6, 7]. The author concludes that no winter growth can be observed in red clover under Moscow conditions. The author succeeded in proving experimentally that populations of red clover of northern origin which have a longer period are more frost-resistant.

In an investigation of the dormant state, the question of change in the physicochemical properties of protoplasm in buds and roots during the fall-winter-spring periods is of considerable interest. One of the fundamental determining quantities in characterizing the physicochemical properties of constituent parts of cells and tissues is the isoelectric point (IEP) [8]. For characterizing oxidation-reduction reactions in the cell, the value of the oxidation-reduction potential (rH) is used [9]. Ellengorn and Svetozarova [10, 11] showed that investigation of the physical properties of cell structure makes it possible to discover their physiological variability.

METHODS

Investigation of the dormant state was carried out in red clover plants, grown under commercial condition in the fields of Sovkhoz "Istok" of the Aramil'sky district of the Sverdlovskaia Region.

The samples for the analysis were taken in the fall-winter-spring period of 1951-1953 from plants of the first and second year growth. Samples were taken every two weeks or, during very changeable physiological conditions (in the spring), every week. Plants were washed free of soil and analyzed.

The state of dormancy was determined by the method of Genkel and Okina [4] on free-hand logitudinal sections, of buds and cross-section of roots.

The isoelectric point of protoplasm proteins was determined on material fixed in alcohol according to methods described by Ellengorn and Svetozarova [11]. Hydrogen ion concentration (pH) was determined by neutral red vital staining, and oxidation-reduction potential (rH) by toluidine blue [9, 10]. The presence of starch in root tissues was determined by the iodine reaction (Lugol's reagent). All of these indicators were used for the following tissues: phelloderm, secondary bark, phloem, xylem, pith rays, and pith on transverse free-hand sections of roots.

Investigations have shown that the dormant state in red clover plants set in toward the end of December, and that the plants remained dormant until the end of March.

In the December sample a considerable number of cells with clearly expressed convex plasmolysis were observed in the apical meristem of the clover bud, but cells with concave plasmolysis were observed in some

plants. In succeeding samples (January-March), concave plasmolysis was not observed; only the convex form of plasmolysis was seen, in which the protoplasm was more rounded than in the December 9 sample, and was almost entirely separated from the cell wall; this was observed not only when sections were placed in a molar sucrose solution, but also in preliminary staining of sections by I + KI. In the March 17 sample, convex plasmolysis was also observed, both on the day on which the sample was taken and after plants were left overnight in water at 18-20° in the laboratory. In the April 6 sample, no convex plasmolysis was observed. Only individual cells with a hardly-noticeable rounding of the protoplasm were seen, but concave plasmolysis or a hardly-noticeable separation of the protoplasm from the cell walls was clearly seen in some cells.

In April and May, no convex plasmolysis was observed. From the above one may conclude that the plants of red clover broke their dormancy in the end of March or the beginning of April.

On the basis of these investigations, it has been established that no changes in the pH of root tissues was observed in the fall-winter-spring periods, but the effect of age is noticeable. Almost all of the clover tissues in the first year of growth have a pH of 5-5.5. In the second year of growth the pH moves toward neutral – from 5.5-6.5. Such changes in pH were observed in the cells in the phelloderm, secondary bark, phloem, xylem, and pith. Pith rays had a pH of 5-5.5 in the first or second year, without any changes because of age (years of growth). Cambium has the same value (pH = 7), both in the first and second year. In the second year, some cambium cells do not take neutral red staining, and part of them – and sometimes a large part – remain unstained.

The oxidation-reduction potential reflects inner changes which take place in the root tissues of red clover plants in the period of spring growth.

The cell contents of phelloderm, secondary bark, phloem, pith and pith rays in the end of the summer, in the fall, and in the winter are stained a pink-lilac color by toluidine blue (reducing regime, rH ≅ 12). In the spring (April-May), the cells of these tissues were stained a blue or blue-green color (oxidizing regime, rH < 14 and rH > 14). Xylem has an oxidizing regime (rH < 14) for the whole year, which changes in the spring, in April and particularly in May, to a still higher oxidizing value (rH > 14, stains green). Throughout the whole year, cambium has a reducing regime (rH ≅ 12); only in the beginning of April is an insignificant shift towards a more oxidizing regime noticed (pH ≅ 13). The changes in value of the oxidation-reduction potential of root tissues in red clover coincide with the period when the plants begin to grow actively. By this time, the plants had 2-3 buds with 1-2 developed but not expanded leaves, and young rootlets on the root (closer to the base of the root). An oxidizing regime was observed until the plants formed well-developed rosettes of 14-15 leaves, namely until the second half of May.

Determination of the isoelectric point showed that the value of pH IEP of protoplasm in all root tissues of red clover plants is changed according to the physiological state of the plants. Thus, the phelloderm cells, in different samples taken in January and February, had a pH IEP of 2.6-3; in those from the February 16 sample, a shift took place to 0.1-0.14. This value of pH IEP was observed later in spring-summer-fall samples. The pH IEP of the secondary bark in January-February had a value of 1.8-3.6 (in various samples). From February 16, values of pH IEP of the secondary bark underwent exactly the same shift as those of the phelloderm (1.0-1.4). A general shift of pH IEP occured in pith rays and pith. In winter samples of these tissues, pH IEP had a value of 2.6-3.8, and from February 16 the value moved toward the acid side (1.0-1.4). Phloem and xylem in winter samples prior to February 16, had a pH IEP of 2.6, and from February 16 the pH IEP value shifted to 1.0-1.4.

In the winter samples of cambium cell protoplasm, the pH IEP was 1.8-2.6 (in various samples) and in spring-summer-fall it was 1.0-1.4.

Cambium cell protoplasm is distinguished from other tissues by the greatest absorption of the basic stain (methylene blue), i.e., the IEP is shifted to the more acid side. This may be explained by the fact that the cambium cell content is more acid than in other tissues, as it contains more nucleic acids [12].

The shift in pH IET of all clover root tissues from February 16 to the more acid side, which remained without change in almost all spring-summer-fall samples, indicates the increase of life activity in the protoplasm of these tissues. These shifts are observed significantly earlier than one can observe the breaking of dormancy of the plants by the plasmolytic method.

We also studied the accumulation and distribution of starch in tissues of the red clover root. Rapid starch accumulation takes place in the second half of the growing period, particularly in July.

Throughout all the winter period, starch occurs in root tissues almost without any changes. Starting in March, starch gradually begins to disappear from the cells of the pith and pith rays, and only in April and May do the starch reserves begin to diminish in the cells of the secondary bark. By May 15, starch almost completely disappears from the cells of secondary bark and pith, and only traces of starch are left in the pith rays.

Various root tissues accumulate starch in various ways. The secondary bark gets filled with starch prior to all other tissues. All cells of secondary bark are completely filled with it, and this state prevails throughout the winter. In the spring, the starch disappears; it disappears from the cells of secondary bark last of all. This takes place in the end of April or the beginning of May.

In the summer, starch appears in the pith rays first of all, but it does not accumulate there, and fills the cells of the secondary bark. After this, as the cells are completely filled with starch, it begins to accumulate both in pith rays and in pith. In the spring, starch begins to disappear from the pith rays prior to any other tissues; however, the pith rays are the last to be completely free of starch. Thus, on May 15 one may still observe some starch in the pith rays, while only traces of it are found in the pith and secondary bark. Starch accumulates in the pith simulaneously with the pith rays (July), and disappears later. Thus, in March the amount of starch is considerably decreased in the pith cells, and by the second half of May it completely disappears.

Of all root tissues, only the cambium does not accumulate starch.

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ON PARTICULATION IN HERBACEOUS PERENNIALS

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The term "particulation" was first used by Vysotskii [1] in 1915.

Particulation represents a special form of vegetative reproduction in which the mother plant divides into several parts capable of independent existence.

The phenomenon of particulation has been described a number of times in the literature. Thus, for example, Jost [1] observed segmentation of the root of aconite, gentian, and other plants. Vysotskii [1] had noticed particulation in some plants of the buttercup, poppy, buckwheat, and composite families. Raikova [2] described the process of formation of "witch brooms" in teresken* in a mountainous desert of Pamir. Radkevich and Shubina [3] have studied the process of particulation in xerophytes of the Betpakdala desert. The phenomenon of particulation in some species of wormwood and boialych, ** both desert inhabitants, was noted by Shalyt [4, 5].

Thus, particulation is comparatively widely distributed in nature, and is observed in many plants. The phenomenon of particulation was first described by Jost in 1890, but the term "particulation", as described above, was used considerably later. According to the opinion of Jost [13], there occurs the death of tissues which are in direct connection with annual organs – leaves, flower-bearing organs – and which are the extentions of these organs in basipetal directions. When the flower-bearing organs die, the cambium of both sections of the axis which leads to those organs ceases its activity, but the bundles of perennial buds continue to grow. The dead tissues are separated from the living tissues by strips of periderm.

The phenomenon of particulation was much more thoroughly investigated by Vysotskii [1], who showed that particulation may take place in various ways in various species. Thus, for example, the segmentation of fibrous-rooted plants, such as those of the buttercup family (Ranunculus polyanthemos, R. acer, Clematis integrifolia, Caltha palustris), according to Vysotskii takes place in the following way. The middle parts of maternal tissues die out, and the lateral stems, connected with the corresponding lateral roots, remain alive. Such sections may be planted separately and grow well. A similar particulation has also been noted in adonis (Adonis wolgensis).

Particulation in anemone (Pulsatilla patens) can be explained differently according to Vysotoskii. With age, the lateral root growing out of the main root becomes stronger than the extension of the main root. The axial stalk, lacking nutrition, loses its tip and depth extensions. Thus, it rots in the middle, forming a tube from which stem branches grow upward, and root branches grow downward. The tube begins to break, to rip, while the walls of this tube become the unifying parts of the lateral stems with lateral maternal roots. The same form of particulation occurs in celandine (Chelidonium majur).

In tap-rooted plants, as in wormwood (Artemisia iodora) and Kurchavka (Atraphaxis spinosa), for example, in which the tap root does not get shortened by death of the tip, particulation, according to Vysotskii, takes place in the following way: a very uneven growth of cambium takes place, resulting in a star-like shape of the stem in transverse section. All rays of this star, running lengthwise along the stem, swell more and more. Then the stem breaks up into long fibrous parts tightly intertwined into a long cable, which may be disentangled.

* A member of the goosefoot family found in the steppe regions of USSR.

** Transliterated from Russian.

Fig. 1. Roots of tadzhik dandelion. A) Root before particulation; B) beginning of particulation in the upper part of the root (photograph by A. S. Babkin).

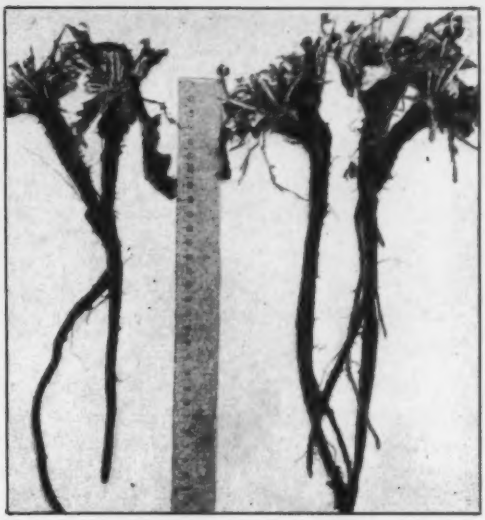

Fig. 2. Separation of particles in tadzhik dandelion (photograph by A. S. Babkin).

Fig. 3. Root of the tadzhik dandelion with clearly-expressed particulation (photograph by A. S. Babkin).

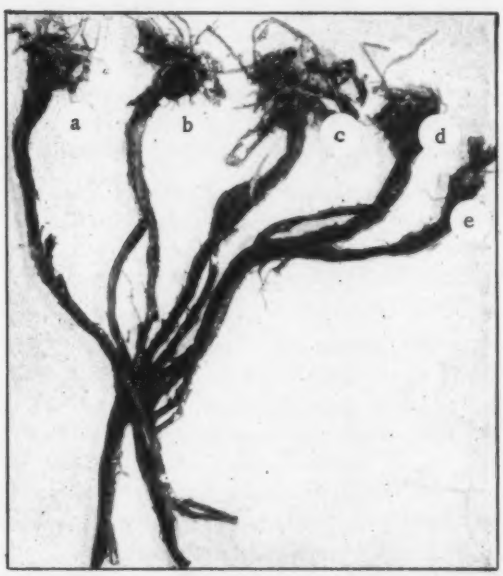

Fig. 4. The root shown in Figure 3 after disentanglement, A,B,C,D) particles (photograph by A. S. Babkin).

Both Jost and Vysotskii, observing the breakdown of plants into particles, have come to the conclusion that the purpose of particulation is a function of age, and does not depend on external environmental conditions. Both investigators had observed the phenomenon of particulation in mesophytes. The structural basis of particulation in xerophytes was laid by Radkevich and Shubina [3], who established the fact that in many perennial herbaceous xerophytes the same type of particulation occurs as is found in mesophytes. These authors also consider that particulation of these plants takes place without a direct effect of variation in environmental conditions. They also consider age to be the decisive factor.

However, Radkevich and Shubina [3], studying particulation in shrubs and semi-shrubs, come to the conclusion that the same process of particulation takes place as a result of severe climatic conditions and does not depend on the age of the plants. Further, they point out that "the more continental is the climate, and the sharper are the climatic contrasts between the spring period of maximal precipitation and summer drought, the earlier particulation begins in a given species, and the more rapid is its development."

The observations of roots of rubber plants have shown that in a given case there is definite connection between environmental conditions of plants and their capacity for particulation.

Thus, for example, Lipshits [6] had pointed out the irregular anatomical structure of kok-saghyz roots, which grows in the outskirts of Tian'-shan'. Litman in 1949 (as quoted in Neiman [7]) had established that in natural outgrowths whole boundaries occur, where kok-saghyz reproduces vegetatively by breaking down into independent roots.

Rudenskaia [8] has shown that in vigorous growth of roots in plantation plants of kok-saghyz, there is a tendency to form many vascular bundles instead of one bundle, and that each bundle has a clearly-expressed root structure, with woody tissue pressed towards the center surrounded by cambial tissues and concentric cirlces of lacteal tissue. Since such structure of the root leads to the formation of a large number of points where new lacteal organs are formed, this leads to the rubber-producing capacity of such roots, as was shown by the studies of Rudenskaia [8] described above.

Bulgakov [9], while working with selected varieties of kok-saghyz, had established that new biological properties connected with changes in anatomical structure of the root were developed in some forms. Change of the anatomical structure of the root begins as early as the first year; however, at this time the process takes place inside the root. Later, in the second year and particularly in the third year, further differentiation and separation of tissues takes place in most plants. Inside the root, a cork tissue is formed around every woody-bark system. The general cork-rubber case splits, the bark parenchyma dies out and new structures come out in the form of separate roots bearing rosettes of leaves. The author justly holds this a peculiar method of vegetative reproduction of kok-saghyz, as the maternal plant is transformed, after a certain period of time, into a group of separate plants, i.e., into a natural cluster.

We purposely described the statements of various investigators about the nature of plant particulation in such detail so as to show that in this field there is no single opinion, and that many aspects of the particulation process are not clarified. This was the basis of the investigation of the process of particulation in a new species of dandelion (Taraxacum tadshikorum Ovcz.), the biological characteristics of which we studied in greater detail [10, 11].

The particulation process in tadzhik dandelion was studied under natural conditions of the plant's habitat. While digging out roots in the Sil'bursai boundary (the southern slope of Gissarskii Ridge, altitude 1900 m above sea level), we have found roots without any external signs of particulation (Fig. 1,a) and roots showing the beginning stages of particulation (Fig. 1,b). We also found roots with a clearly-expressed process of particulation (Fig. 2). As can be seen in Fig. 1,b and Fig. 2, the splitting of the root may take place both in the upper part (Fig. 1,b), and at both ends (Fig. 2). Consequently, the concepts of Radkevich and Shibina [3] concerning the fact that the phenomenon of particulation represents the splitting of the upper part of the root cannot be applied to all plants, particularly not of the tadzhik dandelion. Because of this, we are inclined to concur in the opinion of Shalyt [5], according to whom particulation is not connected with separation of any particular parts of the plant, but may begin in various places, most often from above.

In Fig. 3, a root of tadzhik dandelion is shown in which particles, as Vysotskii [1] pointed out, are "interwoven into a cable, which may be disentangled." Disentangled to separate parts (or particles), the root is shown in Fig. 4, where some particles (a) may be easily separated from the maternal plant without harming the latter but some are still connected, relatively securely, in the center of the root.

Since an anatomical picture of the particulation phenomenon in the tadzhik dandelion was given in a paper published earlier [12], there is no need for a detailed description. We shall mention only the fact that the phenomenon of particulation is preceded by certain regular changes in anatomic structure of the root.

Thus, particulation in the tadzhik dandelion takes place according to the type described by Jost and Vysotskii for mesophyte plants, or according to the type studied by Radkevich and Shubina in many-axiate rosette

plants. However, the authors mentioned above consider that only age has an effect on the process of particulation of these living organisms. Our observations on the tadzhik dandelion have shown that not only does age affect the breaking-down of the maternal plant into particles, but that the breakdown is also affected by characteristics of the sharply continental climate in which tadzhik dandelions grow.

The sharp climatic contrast affects the depth of immersion of the base of the root in the soil. The more severe the environmental conditions (high temperatures, low humidity of air and soil), the deeper the root is immersed in the soil. And it is this immersion of the root base in the soil, and the shortening of the root which takes place during immersion, which leads to the particulation of tadzhik dandelion. The more shortened the root, the faster the splitting of the maternal plant into independent individuals – particles – takes place [12].

In conclusion I should like to express my sincere gratitude to Doctor of Biological Sciences, Professor Alexander Arkad'evich Prokof'ev for help in preparing this paper for publication.

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COLLOID CHANGES IN POTATO PLANTS IN CONNECTION WITH THEIR FROST RESISTANCE

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Questions of resistance of plants to low temperatures are of great interest to most investigators of plant physiology. One of the most interesting and important questions concerns the development of methods to increase plant frost resistance.

The present paper was an attempt to study some colloidal-chemical properties of plant protoplasm during an artifically-produced increase of their frost resistance. The potato variety Imandra, adopted for the Murmanskaia area, was used as an experimental object. As is known, the potato is very sensitive to relatively light frost (from −2.5 to −4°). Under the conditions of the Murmanskaia area such frosts may occur on any day of the summer months. Because of this, the increase of potato frost resistance becomes a problem of great practical importance. In the experiments which were carried out in the Polar-Alpine Botanic Gardens, Kol'skii Affiliate of the Academy of Sciences USSR, we have succeeded in increasing potato frost resistance somewhat (by 1-1.5°) by means of a pre-planting treatment of tubers (for 24 hours) and by treatment of its leaves with a 0.25% solution of ammonium nitrate (during spraying of the leaves, the salt concentration reached 1.5%).

In connection with these experiments, we determined several colloidal-chemical properties of the protoplasm of potato leaves. These characteristics were the amount of coagulant of hydrophilic colloids in protoplasm and the bound water content. Vetukhova [1, 2] noted that one of the methods in studying frost resistance of plants is the study of the colloidal state of their protoplasm. According to that author, the less resistant system coagulates easier under the influence of coagulating agents and gives a greater amount of coagulum. The experiments of A. A. Vetukhova have shown that the lowered frost resistance of plants corresponds to an increased amount of coagulum particles in hydrophilic colloids. The author points out that the qualitative state of hydrophilic colloids plays a great part in the phenomenon of frost-resistance. In frost resistant plants, the amount of hydrophilic colloids is increased but the amount of their coagulum particles becomes smaller.

In our experiments, we determined the effect of ammonium nitrate on the resistance of the colloid system to coagulation in potato seeds, resistance being measured by the size of the coagulation amount of the hydrophilic colloids of the protoplasm in the triple solvent (water, alcohol, ether), according to the method of Dumanskii [3]. In characterizing the coagulation of the colloid system, four degrees were distinguished: 1) full coagulation—compact precipitate and completely transparent supernate; 2) incomplete (partial) coagulation—flocculent precipitate, supernate not entirely transparent; 3) turbidity—coagulation is only beginning and precipitate does not fall out; 4) stratification—no precipitate occurs and stratification of sol takes place.

The results of these experiments were plotted on a triangular diagram—an equilateral triangle. On the diagram we plotted only the zone of complete coagulation of hydrophilic colloids in the protoplasm (Figure 1). The determination was carried out in the stage of flower-bud formation and flowering.

As can be seen from the data given, under the action of NH_4NO_3 the amount of coagulum of protoplasmic colloids is decreased, particularly at the stage of flower-bud formation. The decrease of coagulation amount of hydrophilic colloids of the protoplasm in the phase of flowering is connected with aging of the potato plants [4].

TABLE

Bound Water Content in Potato Leaves as Affected by Ammonium Nitrate (in %)

Experimental treatments	Vegetative phase		Flower-bud formation		Flowering	
	1954	1955	1954	1955	1954	1955
Control	25.6	31.5	28.9	31.4	24.5	25.9
Ammonium nitrate	17.5	28.1	31.2	30.8	23.8	28.5

The decrease in the amount of coagulum after complete coagulation in the potato under the effect of ammonium nitrate was also observed after spraying the leaves with a solution of this salt (Figure 2).

Thus, ammonium nitrate results in a decrease in the amount of complete coagulation of hydrophilic colloids in the protoplasm, which indicates an increase in resistance of the colloid system in potato under the action of this salt. And if one considers that the amount of the coagulum depends on the amount of hydrophilic colloids in the protoplasm, it is then possible to consider that a large part in the phenomenon of frost-resistance is played not by the amount of hydrophilic colloids, but by their qualitative state. From this, it follows that the smaller the value of colloid coagulant in plant protoplasm, the greater is their resistance to lower temperature.

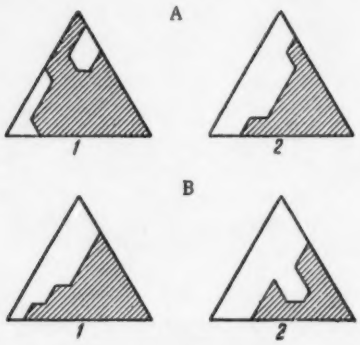

Fig. 1. Amount of coagulum after complete coagulation of hydrophilic colloids in potato leaf protoplasm as affected by ammonium nitrate. A) Stage of flower formation (August 5, 1955); B) stage of flowering (August 19, 1955). 1) Control; 2) NH_4NO_3.

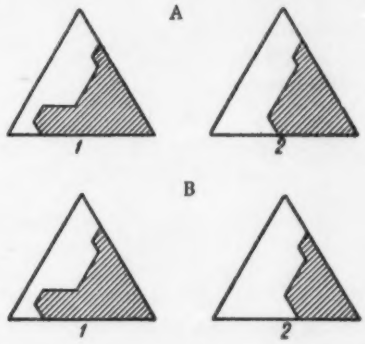

Fig. 2. The amount of coagulum after complete coagulation of hydrophilic colloids of protoplasm in the potato after spraying with a solution of ammonium nitrate. A) After fourth spraying (August 25, 1955); B) after fifth spraying (August 30, 1955). 1) Control; 2) NH_4NO_3.

Most investigators link the great hydrophilism of protoplasmic colloids with an increased percentage of bound water in the protoplasm [5, 6, 7]. In addition, greater frost resistance of plants is usually determined by a greater percentage of bound water. Determination of the bound water content in our experiment, carried out by the Dumanskii method, has shown that the amount of bound water is decreased particularly in the vegetative stage, under the effect of ammonium nitrate, i.e., in the potato which is more frost resistant. It it true that in some cases the bound water content in experimental plants is somewhat greater than in the control, but this is not the rule, and it can be asserted that ammonium nitrate is more effective in decreasing the bound water content than in increasing it.

After spraying potato leaves with a solution of ammonium nitrate, a decrease in bound water content was also observed; in 1954, there was 31.2% bound water prior to spraying and 27.9% after spraying; in 1955, the bound water content was 28.5% prior to spraying and 27.6% after spraying.

Thus, our data agree with the date of Vetukhova [1, 2] concerning the smaller amount of coagulation of hydrophilic colloids in the protoplasm of more frost-resistant plants: while ammonium nitrate increased potato frost-resistance, it decreased the value of the coagulation range of hydrophilic colloids in potato protoplasm.

Bound water content in potato plants with increased frost resistance, is in general, lower than in controls. This indicates that a large percentage of the bound water in the plant does not always correspond to greater resistance of the plant to lower temperatures.

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VARIABILITY OF WET PROTEINS IN CLOVER LEAVES IN CONNECTION WITH THE POPULATION, AGE, AND SITE OF GROWTH

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Clover is the best component in grain-legume rotations in regions of sufficient humidity, as well as being one of the forage plants which supplies proteins. K. A. Timiriazev wrote that there are not many discoveries in history that were as beneficial for humanity as the inclusion of clover, and legumes in general, into crop rotation—so remarkable is its effect on the increase in the productivity of the agriculturalist's labor.

A very important regularity was established by the investigations of Ivanov [1-3] and his students. Legumes planted under the usual conditions of cultivation and with normal development of nodule bacteria do not change their percentage of protein in various climatic zones when NPK is applied to the soil; the difference in soil and fertilization affect not only yield but also nitrogen content. On the other hand, when planted in agricultural regions unusual for them, legumes (soybean, lupine, chick-pea) vary markedly in their nitrogen content; NPK in this case, as in the innoculation of seed, greatly affect the nitrogen content in seeds. In this connection, it was very interesting to follow the change in nitrogen content in clover as affected by edaphic factors, in relation to the place where it grew, its age, and its population or variety. This makes it all the more clear that "the problem of amount of protein in forage plants is difficult, and has not been solved up to the present time" [2].

METHODS

The experiments were carried out on the dried peat bog of the Station of the Rovenskaia Region and on the mineral soil of the Botanical Garden of the I. Franko L'vov State University.

Peat bogs of the Sarnenskaia Station are typical of the Poles'e area of the Ukrainian SSR. The peat layer in the fields of the station reaches several meters in depth. On the other hand, the soil of the botanical garden was sandy loam, containing 17-19% of physical clay.* The soil water level in both cases was 60-100 cm from the surface. The peat bogs of the station are of the lowland type, and are rich in phosphorus and nitrogen. The organic remains of peat make up 87-91%, and there is 2.9-3.1% of humus in the mineral soil of the botanical garden; the pH of the peat is 5.1-5.4, and that of the mineral soil is 6.9-7.1; all of this indicates the sharp difference in environment and other conditions in which the experimental plants were grown. 109 samples of various species of clover were investigated, of which 84 were meadow species.

The samples planted represented the best forms of the natural meadows of the forest region, as well as old Russian, local and and selected clovers, originated in BSSR Poles'e, Bashkirskaia ASSR, Pskovskaia, Molotvskaia, Leningradksaia, Chernigovskaia, Kirovskaia, and other regions, and also Italy, Denmark, England, Germany, USA, and Sweden. The planting was made without a cover crop, and cultivation consisted of the periodical pulling of weeds.

We took for analysis plants of one phase—flowering, and determined the percentage of nitrogen in these by the macro-Kjeldahl method. Before analysis, the leaves were dried out in an oven and their moisture was determined.

Results of analysis and discussion are presented for a meadow clover.

*As in original.

Results of Analyses and Discussion

As we shall show, the protein content in meadow clover leaves depends first and foremost on the age of plants. Plants of the second year's growth, as a rule, contain a larger percent of wet protein than first year plants. The picture of these changes is clearly seen from the variation in protein content in clover leaves of first year plants.

The variations in the protein content in meadow clover leaves with relation to plant age turn out to be the following:

On Mineral Soil

Percentage of wet proteins	20 – 22	– 24	– 26	– 28	– 30	– 32	– 34	– 36	– 38
Amount of clover samples, first year plants (in %)	21	17	33	23	6				
The same, second year plants	5	9	30	17	21	13	5		

On the Dried-Out Peat Bogs

Amount of clover samples, first year plants (in %)	–	14	19	21	31	15	
The same, second year plants	–	—	–	14	14	25	47

In addition, the data obtained indicate also that the number of samples rich in protein was greater on the peat bog than on the mineral soil. Thus, on the mineral soil 38% of clover samples contained up to 24% of wet protein in the leaves, while on the peat bogs, only 14% did so; correspondingly 6% of the samples on the mineral soil had 28-32% of wet protein, while 46% of clover plants on the peat bog contained that amount of protein. Thus, the synthesis of protein substances in the leaves of the same experimental clover varieties is increased when they are grown on the peat soil, as a result of which many clover varieties, poor in protein content on mineral soil, became rich in leaf protein when grown on dried-out peat bog. In our opinion, this very interesting regularity should be considered during directed cultivation, development of clover varieties and their evaluation for forage and biochemical properties. The latter may be illustrated by the fact that the carotene content in clover leaves is changed almost in the same way as the wet protein content. In the peat bogs, the meadow clover leaves contain more carotene than do the leaves of samples grown on mineral soils.

TABLE 1

Protein Content (in %) in Leaves of Meadow Clover in Relation to the Soil on Which It Was Grown

Sample number	Soil		Sample number	Soil	
	Mineral	Peat		Mineral	Peat
LGU-29	28.13	36.88	LGU-19	23.75	28.75
LGU-61	18.75	31.25	LGU-22	20.00	29.37
LGU-35	24.37	27.50	LGU-31	22.50	31.25
LGU-30	22.50	29.37			

The development of this characteristic (high protein content in leaves) in meadow clover plants is very clearly seen during the second year of growth. For example, when clover was grown on mineral soil, 38% of the first year samples contained up to 24% of protein in the leaves and only 14% of the second year plants contained that amount of protein; 6% of the samples of the first year clover plants contained more than 30% of protein, while 39% of the second year plants contained that amount. Attention can be drawn to the fact that in the first year there were no samples in which the leaves contained more than 30% of wet protein.

An almost similar but a more contrasting picture had been observed during clover growth on the peat bogs. Thus, 33% of the first year samples contained up to 26% of wet protein, but in the second year there was not a single sample in which the leaves contained less than 28% protein; 47% of first year clover samples contained 36-38% of protein in their leaves. Such a high leaf protein content was not found in any clover sample grown on the mineral soil. The strong effect of peat soil on the leaf protein content is also indicated by the data of Table 1.*

* In the paper we retain the sample numbers corresponding to the catalogue of L'vov State University (LGU).

TABLE 2

Wet Protein Content in Meadow Clover Leaves in Relation to the Year of Growth

Catalogue number of samples	Protein content, %		Catalogue number of samples	Protein content, %	
	First year of growth	Second year of growth		First year of growth	Second year of growth
LGU-52	21.25	28.12	LGU-21	20.00	28.75
LGU-45	27.50	24.37	LGU-18	27.50	29.44
LGU-35	24.37	23.44	LGU-19	26.85	25.62
LGU-30	22.50	30.00	LGU-39	24.06	25.56
LGU-46	25.62	30.62	LGU-19	25.62	27.19
LGU-19a	23.75	31.87	—	—	—

Such a significant response of clover plants to soil conditions indicates great possibilities of protein increase and improvement of feed value of legume plants by means of applying suitable doses of fertilization and by other agrotechnical methods.

Returning to the question of the effect of plant age on protein content in clover leaves, we refer to Table 2.

It was shown above that protein content in clover is greater in the plants of the second year of growth as compared to the plants of the first year of growth. As can be seen in Table 2, this regularity may have exceptions, or it may manifest itself differently in different populations. Thus, for example, in sample LGU-52, LGU-21, LGU-30, and others, the leaf protein content was greatly increased in the second year of growth; there are also populations (not many, it is true), in which the leaf protein content was almost the same in the first and second year of growth, and was sometimes even smaller in the second year of growth (LGU-45, LGU-35, LGU-19, LGU-39). The latter is altogether regular, if different degrees of inheritance conservation as well as different heredity of the population is taken into account. In addition, this confirms the regularity which we have found of various rhythms of growth in different varieties and populations of clover in the first and second year of growth [4]. The different manifestations of yield production of various clover populations are, in our opinion, adaptive towards conditions of cultivation: when maximum yield is observed in the first year of growth, the variety is adapted to cultivation without a cover crop; when, however, the yield of green matter is small in the first year of growth, and in the second year this characteristic is well developed, the populations are adapted to cultivation with a cover crop. Unfortunately the investigators did not pay attention to this aspect, and therefore obtained conflicting results when using cultures with or without cover crops.

The clover populations were also different with relation to the leaf protein content in the first and second years of growth. The investigation of the dependence of protein content on yield has shown that the protein content is a relatively discrete characteristic. Cases are frequent in which high protein content occured in a high-yielding variety or population of clover, as well as in low-yielding ones. This underscores the necessity of carrying out selection and evaluation of selected material for biochemical properties while considering the dynamics of development of the plants, and the characteristics and purposes of cultivation, so as to establish the most favorable period for development of this or that characteristic and to establish a basis for the agrotechnology of the variety. In our case, it seems, the most favorable factor for the increase of leaf protein content for a number of populations would be a second year of growth, or harvesting in the first year of growth, if the protein content is maximal in the first year of clover growth.

Received September 8, 1956

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METHODS

SUPPLEMENT TO DEVELOPMENT OF AMINO ACIDS BY ISATIN ON PAPER CHROMATOGRAMS

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In 1956 we published [1] a method of developing amino acids with isatin, which gives different characteristic stains with the spots on paper chromatograms. This variation in stains greatly facilitates identification of individual amino acids, and is a great help in avoiding the mistakes which arise when chromatograms are developed with monochromatic ninhydrin, in which case identification is made mainly on the basis of spot location (R_f). The further development of an improved isatin reaction is presented in this paper.

For the development of the chromatogram, the two following reagents are prepared:

1. One g of isatin is dissolved in 100 ml of 95-97% of ethyl alcohol with heating, and 1 ml of concentrated acetic acid is added to the solution. The solution is stable for several weeks when kept in the dark.

2. Ten ml of 20% soda solution is added to 40 g of dry sodium silicate in a beaker, and the mixture is heated to 60-70°C. As the mixture is heated and stirred, it becomes at first paste-like, then turbidly liquid, and finally transparent. This solution can be kept indefinitely in a rubber-stoppered bottle. Sodium silicate, which occasionally precipitates during storage, can be again brought into solution by heating the flask to 50-60°. The concentration of the solution can also be determined by titration with phenolphthalein; 7 to 8 ml of 1 N HCl should be required per ml of sodium silicate solution.

Chromatograms of amino acids are at first impregnated with the first reagent and are thoroughly dried for 30 minutes at room temperature. Then they are heated, as was described in the previous paper, between two previously-heated plates of glass at a temperature of 90-100° for, correspondingly, 20-10 minutes. After heating, the chromatograms with developed amino acid spots are brushed with a solution of sodium silicate, the excess of which is removed with filter paper. During this treatment, the yellow background of the chromatogram at first turns red; however, very soon this color fades and disappears, so that, in the end, the brightly-colored spots of amino acid are seen on a colorless or slightly yellow background. Drying of chromatograms after treatment with sodium silicate should be carried out as rapidly and completely as possible, best of all in a stream of warm air, however at a temperature not higher than 40-50°, as at a high temperature the yellow background may reappear.

Chromatograms treated in this way must be kept from moisture, by placing them in moisture-proof boxes, bottles, or desiccators with dry calcium chloride, or by rolling them tightly into compressed oilcloth. If kept in a humid atmosphere, chromatograms fade considerably in a few days.

As distinguished from the method I described in the previous paper involving additional chromatogram treatment with magnesium sulfate, in the new method II, with additional chromatogram treatment by sodium silicate, the sensitivity of the isatin reaction for most amino acids is increased to the level of the ninhydrin reaction.

The only exceptions are proline and oxyproline, the blue spots of which disappear after treatment with sodium silicate; because of this, the spots of these amino acids must be outlined with pencil prior to this treatment.

TABLE

Color of Amino Acid Spots on Chromatograms After Development with Isatin According to Methods I and II (see text)

Compound	Method I	Method II
α-Alanine	Violet to violet-blue	Violet to violet-blue
β-Alanine	Violet to violet-blue	Violet to violet-blue
α-Aminobutyric acid	Red-violet to violet-blue	Violet to violet-blue
Arginine	Red to violet-red	Violet-red
Asparagine	Red to violet-red	Violet to blue
Aspartic acid	Violet to blue-violet	Violet to blue-violet
Valine	Red	Red
Histidine	Grey-pink to grey-green	Grey-violet to grey-green
Glycyl-glycine	Red	Brown
Glycine	Red	Red
Glutamine	Red	Red-violet
Glutamic acid	Red to violet-red	Red-violet to violet
Glutathione	Red	Violet-red
Isoleucine	Red	Red
Leucine	Red to violet-red	Red to violet-red
Lysine	Violet-blue to green	Red to violet-red
Methionine	Violet-red to violet	Violet to blue-violet
Norvaline	Violet	Violet
Norleucine	Violet	Violet
Oxyproline	Blue	Blue, disappearing
3,4-Oxyphenylalanine	Turbid-red to dirty violet	Dirty violet to dirty blue
Ornithine	Violet-blue to blue	Violet-red
Proline	Blue	Blue, disappearing
Serine	Brown-red to red	Red-brown
Taurine	Red	Red
Tyrosine	Dirty violet to dirty green	Dirty blue to dirty green
Threonine	Red to violet-red	Yellow-brown
Tryptophan	Dirty pink to turbid green	Dirty violet to dirty green
Phenylalanine	Red-violet to blue	Violet to blue
Cystine	Grey to grey-red	Grey-violet-red
Citrulline	Red	Red
Ethylenediamine	Brown	Red-brown

As can be seen from the foregoing table, the color of the spots of many amino acids may vary to a certain degree, depending upon the temperature, duration of chromatogram heating, purity of the isatin preparation, and quality of chromatographic paper. However, under constant conditions these colors are constant. From the same table, it can be seen that some amino acids, (for example: lysine, ornithine, asparagine, and threonine) give very different contrasting stains with methods I and II, which may be used as an additional criterion for identification of these amino acids. For this purpose, the chromatogram is cut in two, and one half is developed by each method.

Substitution of potassium silicate for sodium silicate in method II leads to the same strong staining of amino acid spots; however, the effect of moisture is greater after use of this silicate, and after storage under room conditions, these chromatograms fade very rapidly. More stable staining of the spots is produced by silicate glue, diluted with 20% soda solution in a ratio of 3:1.

In conclusion, it should be mentioned that, in addition to the papers referred to in our previous paper [1] on the application of isatin for development of amino acids, a new long paper by Saifer and Oreskes [2] has recently been published, in which the authors tested 51 compounds with relation to their reaction with isatin. Their method of applying this reagent is, however, quite distinct from the one described by us.

Received June 10, 1957

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METHODS FOR INVESTIGATION OF THE EFFECT OF LOWERED SOIL TEMPERATURE ON PLANTS

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Up to the present time the effect of soil temperature, especially of lower temperatures, on the growth and development of plants, soil nutrition, and other physiological processes and their yield have been very insufficiently studied. This is explicable, above all, by the difficulty of maintaining the necessary temperature at the will of the investigator, throughout the whole growing period. The maintenance of lower temperatures is, if anything, more difficult than of increased temperatures.

In Russia , experiments studying the effect of soil temperatures on plants have been carried out by Tol'skii and Kossovich in the beginning of the present century. Various types of zinc tanks with water into which the plant containers were placed were used for establishment of various temperatures. Raised or lowered temperatures were obtained for short periods of time by warming the water with a kerosene lamp or by adding ice to the water. The action of temperature on the plants was continued only until boot-formation. The plant containers were covered from bad weather by hothouse frames.

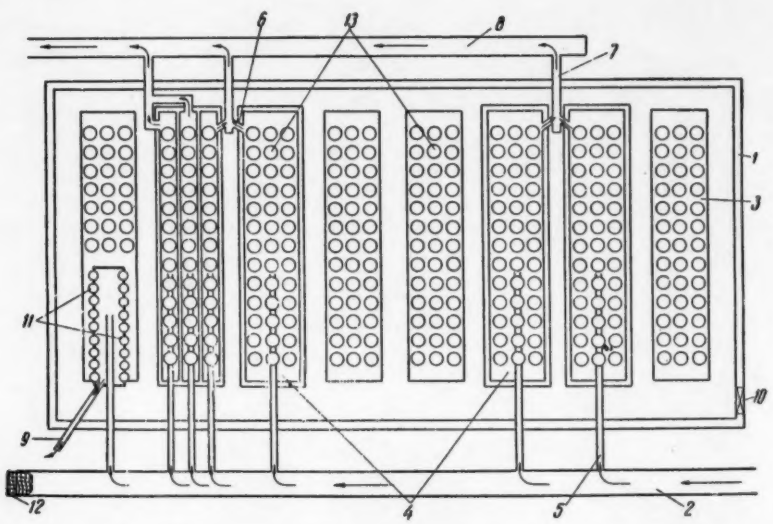


Fig. 1. Diagram of arrangement and functioning of the tanks and benches of the thermo-vegetative house. 1) External walls; 2) the general distributing pipe with plug; 3) bench with containers; 4) tanks with containers; 5) branch pipe for introduction of water into the tank; 6) the location of overflow for the water from the tank into the outflow channel; 7) outflow channel; 8) the main outflow channel; 9) outflow channel from tank for the rider-vessels; 10) doors; 11) rider-vessels in the tanks; 12) the plug of the distributing pipe.

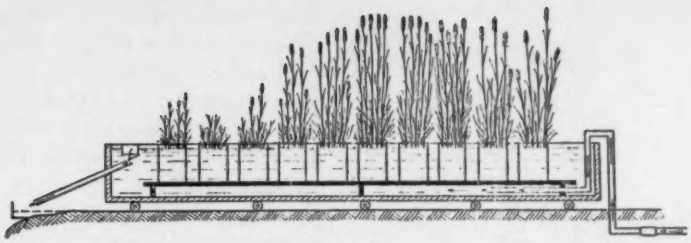


Fig. 2. Diagrammatic section of the tank.

There is the original, but very expensive, method of Radchenko, in which soil and air temperature conditions were maintained under laboratory conditions with the aid of various apparatus. Because of its expense, the Radchenko method did not find wide application in research work.

There is the simpler and cheaper method of Dadykin. He set up a special thermo-vegetative assembly. The cooling of containers filled with soil or nutrient solution has been accomplished by circulation of a refrigerated table salt solution. As a result of the continuous circulation of the cooled solution, it was possible to maintain a soil temperature of 1-3° in the containers. But this method must be applied under conditions of refrigeration. It is still expensive, and demands rather complicated apparatus.

Fig. 3. Diagram of a rider vessel.

While studying the effects of Northern conditions on the growth, development and yield of plants, we encountered the necessity of obtaining lower soil temperatures throughout the whole growing period.

For obtaining lowered soil temperatures, we used spring water. The spring water in the location where we did our work is about 6° in the warmest part of the summer. With aid of this water, it is possible to establish all gradations of temperature beginning with 6° and higher. For the use of the spring water for cooling purposes, in 1953 at the Solikamskaia Agricultural Experimental Station we equipped a special glass-covered thermo-vegetative house, 5 m wide and 10 m long, 2 m high in the lower part of the roof, and 3.5 m high in the higher part. For ventilation, screened windows were placed in the front parts of the house, two frames on each of the two sides can be opened. The tanks and benches are placed inside of the house. The regulation of soil temperature takes place in the plant containers in the tanks, and control containers are placed on the benches.

The arrangement of the tanks and benches and the water supply system is shown in the diagram (Figure 1). As can be seen from this diagram, the spring water goes into the water supply distributing pipe. From this pipe branch off smaller pipes along which water goes into the tanks filling them from below up to a certain level, and overflowing into the outflow channel. The plant containers with soil were placed on special racks in the tanks. The soil temperature in the container is either equal to the temperature of the water or is somewhat higher (by 0.1-3.0° on a hot day). The water enters the tank continuously and overflows, so that exchange may take place in 12 to 15 minutes. The cold water coming from below replaces the upper layers which are beginning to be warmed. The construction of the tank is shown in Figure 2. The tank is made from wooden boards, 50 mm thick. The inside dimensions of the tank are: length—4 m, width—80 cm, height—40 cm. Alongside the tanks are the benches on which control containers are placed. The bench is a table of the same size as the inside dimensions of the tank. Once started, the tank may operate continuously throughout the whole summer. The regulation of inflow and outflow of water will regulate the soil temperature according to the desires of the investigator.

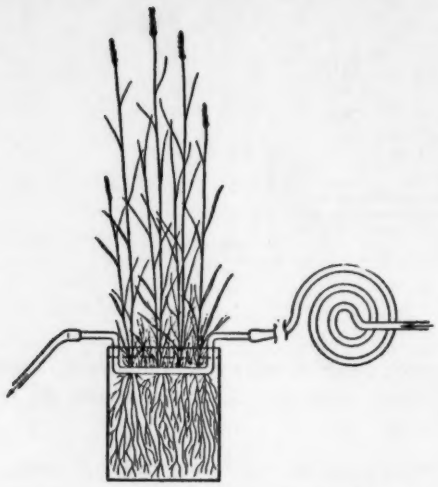


Fig. 4. Diagram of functioning of a snail-pipe.

For establishment of various gradations of temperatures, one of the tanks was divided into three sections. Given a different rate of flow of water in each section, different temperatures of the soil in the containers can be maintained. Thus, in the first section the flow of water is maximal. The water temperature here was within the limits of 6-7° throughout the whole growing period; in the second section the flow of water was decreased. The water temperature of that section was 2-3° higher than in the first section. In the third section, the flow of water was held to a minimum; because of that, its temperature was 3-4° higher than in the second section, i.e., it was 12-14°. The soil temperature in control containers on the bench was 3-6° higher than in the third section. Using such a tank it was possible to have four gradations of soil temperature throughout the whole growing period.

Plant containers made out of plated glavanized iron, painted with oil paint, were used for experiments. Containers were 25 cm high, and 20 cm in diameter.

For studying the plants' capacity to assimilate various elements of soil nutrition applied as various fertilizers at lowered soil temperatures, we constructed rider-vessels on the principle of differentiated nutrition. The construction of rider-vessels is shown in the diagram (Figure 3). As can be seen, a rider-vessel consists of two vessels connected in their upper part. The rider-vessel is placed on the side of a special tank with sides whose upper edge is sharp. As a result, one part of the vessel is located in the tank, the other part is in the air. The part of the vessel which is located inside the tank is constantly exposed to the cold spring water. In it, a lowered soil temperature is established. In the other part of the vessel, the soil temperature depends on the air temperature. The plants are planted as seedlings in such a way that a like number of roots are placed in the cold and the warm part of the vessel. Depending upon the purpose of the experiment, the mineral nutrition elements used may be distributed in various ways between the warm and the cold part of the vessel. Thus, for example, the nitrogen may be applied in the cold part of the vessel, while all others are placed in the warm part, and vice versa. As a result, one may establish which nutritional element is assimilated worst of all at the lower soil temperature, and may determine how to combat this deficiency of the plant.

This method of investigation of the effect of soil temperature on the plant was checked by us for three years. It turned out to be exceptionally effective, convenient, simple, and very cheap.

For study of the role played by the roots and the nodes of boot-forming branches in plant life, we used a snail pipe method. The snail-pipe is essentially a pipe, 1 cm in diameter, which is coiled in a spiral, like a snail, in one plane. The spiral is placed in the container at the level of the boot-bearing node of the plants (Figure 4). The ends of this pipe are bent and come out of the container. The inflow and outflow rubber tubes for water are attached to these ends. The nodes of branching may be cooled, without cooling the roots by flowing water through these snail-pipes. The seedlings are planted between the coils of the snail pipe.

For investigation of the effect of lower temperatures on plants in Iakutsk (under permafrost conditions) we used the method of cold terraces, or as we have called them, thermoterraces. The preparation of a thermoterrace begins in the fall. A flat plot is selected, the upper level of the soil is removed, and trenches 70-80 cm deep and 50-60 cm wide are dug along the edges. Before the onset of frosts, the terrace is watered abundantly and is frozen. Throughout the winter, the snow is regularly removed from the terrace, so that the soil in it may be more deeply frozen (at -45 to $-50°$). In the end of the winter (March), the terrace is covered with peat and a layer of sawdust 20 to 25 cm deep. The trenches are filled with the same material, so as to insulate the main terrace from the penetration of heat. Boards for walking and for preventing compression are placed on the top of this insulating layer. Strips 80 to 90 cm wide are left between these boards. At a certain time the insulating

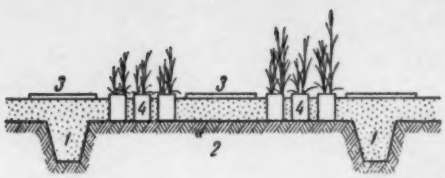


Fig. 5. Diagram of thermoterrace construction. 1) Insulation material (sawdust, peat); 2) frozen soil; 3) boards for walking; 4) plant containers.

layer in these strips is pushed aside, and the plant containers are placed in position. For the warm controls, special benches are constructed in which all the other conditions except temperature were the same as on the thermoterraces. (Figure 5).

With the aid of thermoterraces, it was possible to maintain lower temperatures of 2, 5, up to 10° over the course of 30 to 40 days. Later, the thermoterrace warms up, and the temperature rises above 10°.

The method of thermoterraces can not be considered complete; nevertheless, thanks to that method, we were able to carry out many experiments on the effect of lowered soil temperature, especially in the first period of plant growth. This method can best be adapted in regions which have permafrost or severe winters.

The study of the temperature factor was not interrupted in the winter period, thanks to the construction of thermal equipment in the light laboratory of the Pedagogical Institute. The illumination in this laboratory was furnished by daylight fluorescent lamps and incandescent lamps. For maintaining lower temperatures in the thermal apparatus, cold air entered by suction through a pipe and was directed through distributing pipes into the different sections. Each section was a reservoir made out of sheet iron, 40 x 15 x 120 cm in dimensions.

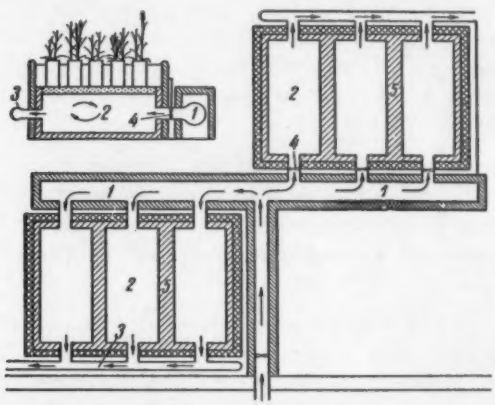


Fig. 6. Diagram of thermal apparatus in the Light Laboratory. 1) Distributing pipe; 2) reservoirs; 3) outflow pipe; 4) valves; 5) insulation of reservoirs.

The inflow of cold air into the main pipe in each section is regulated by valves. The main pipe and distributing pipes were insulated from the surrounding air by felting. Wet sand was placed on top of each section, and on it the plant containers, Petri dishes or pots were placed. As a result, it was possible continuously to maintain temperatures from −3 to 15 or 20°. Combining the methods of thermoterraces and the thermal apparatus, it was possible to carry out the work both in winter and in summer without interrupting the important research, but of course on a different scale.

The methods described may be modified and applied in any place in which it is necessary to study the effect of lowered soil temperatures.

The methods described are cheap and easily available. They may be used for scientific research by plant physiologists, agronomists, soil scientists, microbiologists, plant breeders, etc.

Received May 6, 1957

BOOK REVIEW

A. A. Nichiporovich. PHOTOSYNTHESIS AND THE THEORY OF OBTAINING HIGH YIELDS

Timiriazev Reader, XV. AN SSSR Press, 1956. 94 pages, price 3 r. 65 k

There exist a considerable number of papers devoted to the problem of photosynthesis as a factor in obtaining high yields. In the Soviet literature alone, one may count about ten such essays and monographs (V. N. Liubimenko, E. F. Votchal, L. A. Ivanov, V. M. Katunskii, N. A. Maksimov, A. S. Okanenko, A. A. Nichiporovich). However, it seems that in none of the papers published earlier is there such clarity in the statement and treatment of the questions involved as in the present work of A. A. Nichiporovich. The author set himself the goal of constructing a theory of obtaining high yields, at least to a first approximation. In his opinion, "a theory of obtaining previously planned high and stable yields must give precise information about the following: what is the general potential productivity of the plant; what is the optimal process of yield formation in each plant that interests us; which conditions are required for this optimum; to what degree may various complexes of natural environmental conditions satisfy the requirements of plants; by which ways and methods may the plant's requirements be met to the best degree." It must be admitted that, on the whole, the author reached the goal that he set for himself.

At the basis of yield formation lies the process of plant nutrition, which consists of root nutrition and atmospheric nutrition, or photosynthesis. The author emphasizes that the effect of all other nutritive pathways is determined only by the degree to which they support the main function of the plant—photosynthesis, as the process of formation of organic substances, and that agriculture itself represents nothing but the most wholesome utilization of photosynthesis.

The author begins his discussion of factors of yield production by a consideration of the photosynthetic apparatus (it may be said here that a more acceptable term would have been "photosynthetic organs"). The part devoted to this problem is an abstract presentation of the views that were developed by the author in another, earlier paper. According to these views, yield is seen as the sum of the photosynthetic work of leaves multiplied by the coefficient of photosynthetic efficiency, the pure productivity of photosynthesis, so-called. The author considers very well the complexity of the problem discussed, not only from the quantitative, but also from the qualitative, point of view. It is particularly important that in corresponding periods of growth the distributions of assimilants should be most favorable for the formation not only of biological but also of agricultural yields. Analysis of the materials at hand permits formulation of the conditions for obtaining the highest yield as the following: the best process of growth in leaf area, the longest time for their function, the higher rate of photosynthesis, and the highest value of the coefficient of photosynthetic efficiency, with the best distribution of the assimilants formed, at first toward the formation of the nutrient organs, and then toward the formation of the reproductive and storage organs which compose the main mass of the yield. It seems that it might have been expedient to place in this part the author's consideration of such facts as that during high photosynthesis in koksaghyz, the weight and yield of plants is lower than in the plants with a lower photosynthetic rate (which he mentions in another paper), i.e., a consideration of the significance of the nature of plants with relation to their utilization of assimilants.

In the succeeding section, "Dynamics of leaf area formation as a factor in obtaining high yields," the author, while pausing for discussion of the development of the assimilating surface of plants, emphasizes that the plant's dimensions and the rate of their growth is most of all correlated with the leaf area ($r = + 0.89$), rather than with the rate of leaf function (the latter has very little connection with accumulation of the yield mass). However, the author says nothing about the kind of resources at the expense of which the intense growth of large

plants takes place as compared with that of small plants. Since plastic materials, which are used for the building of plant bodies, arise only in the process of photosynthesis, and since utilization of the assimilants formed in large plants also takes place more rapidly, then, evidently, it should be considered that photosynthesis, too, proceeds at a higher rate in large plants, although we do not take it into account by the methods used at the present time.

In the second part of this section, the author raises a very important question concerning the formation of photosynthetic organs in plants under cultivation and under field conditions. Under these conditions, we are interested in the yield from a unit area, and not the yield of a single plant. Since the production unit is the unit of area, so must we be concerned with the optimal leaf area of such unit, in connection with maximal utilization of solar radiation (for photosynthesis and formative processes) and with those moisture resources which determine the disturbance of the water regime of plants by leaves.

On the basis of a number of contemporary works, the author represents in a graph the utilization of solar radiation under different conditions, in which 20% of the incident energy is reflected by the leaf and the remaining 80% is absorbed in various ways in connection with the leaf surface area. With an increase in leaf area up to 25 thousand m^2 per hectare, the absorption of solar radiation increases almost proportionally to the area, reaching 50-70%. Then the curve of absorption deviates considerably from the curve of increase in area, reaching 75% at 40 thousand m^2. With further increase of leaf area, the functioning of the middle and lower leaves occurs along with a sharp decrease of radiation, which decreases the total balance of the energy utilized. An important circumstance is the fact that if the fully-exposed leaf photosynthesizes at maximum rate even with 50% of complete solar illumination (Timiriazev), then, another reaction is observed in a dense planting, in which the lower and middle leaves are exposed to sharply-decreased illumination: a weakening of illumination is accompanied by a decrease of the photosynthetic function of the unit of planted area. The calculations show that only with a leaf area of approximately 30-35 thousand m^2 per hectare may one expect normal conditions for photosynthetic activity of the lower leaves (in cereals and in plants with edible roots). With further increase of leaf area, the middle and especially the lower leaves begin to use large amounts of organic substances (for growth and respiration) and, especially of water, which, in the final summing up, does not lead to good conditions (except in those cases in which planting is made with the aim of collecting the whole mass of the resulting growth).

Starting with ideal conditions, the author gives sample graphs of leaf area formation for various growing periods. As the formation of assimilating organs means also the formation of transpiring organs, while 98% of all absorbed radiation is used for evaporation, the author considers the energy balance of plants in connection with their water regime, and gives an evaluation of the graphs of leaf area formation in plantings from the point of view of water relations. The author solves the latter question very cleverly, starting with the method of transpiration determination suggested by L. A. Ivanov. Since only the optimal graphs and the optimal conditions of their accomplishment in connection with water relations are considered, then such conditions may exist only in the case in which plants will be provided daily with as much water as can be evaporated in relation to the amount of absorbed radiation. The latter can easily be calculated, given the sum of the incident radiation and reading from the optimal graphs the sum of absorbed radiation in relation to the leaf area formed. Dividing the latter value by the value of the latent heat of evaporation, one can obtain the value of daily transpiration. However, at this point the author completely by-passes the effect of the relative humidity of the air, which is one of the essential factors determining the transpiration rate. Particularly with high relative humidity must evaporation be lower than follows from the recommended graph, while part of the absorbed energy undergoes other transformations.

For facilitating calculation, the author constructed graphs by which the amount of water evaporated may be calculated in relation to the leaf area and the total amount of incident radiation. The examples which he gives agree relatively well with calculations according to the graph. Together with this, the author writes that under conditions of moisture insufficiency the good seedlings often begin to be more injured and more retarded in their growth than do those seedling which had poorer growth and were slower to develop leaf surface. It is necessary, also, to bear in mind a certain correction for drought resistance; the higher the drought resistance, the greater may be the deviation from the graph. It is this circumstance, in particular, that makes it possible for the author to formulate the evaluation of plant drought resistance in a somewhat unusual way: the higher the drought resistance, the greater is the deviation between the amount of water which must be evaporated according to the optimal graph and the actual evaporation observed without harm to growth and photosynthesis. In those cases in which the process of formation of the optimal leaf surface is interrupted, particular care must

be taken for its reestablishment by means of a corresponding agro-technique and method, which will decrease or regulate the use of water by plants.

The author sees further ways to increase yield (after the formation of leaf surface is terminated) in the increase of productivity and of the rate of photosynthesis, as well as in the increase of the coefficient of efficiency and of the harvested portion of the plants. The author constructs optimal graphs of the formation of photosynthetic productivity, and utilizing these, he also constructs graphs of the accumulation of the dry matter. These graphs actually show the possibility of obtaining various yield values in relation to various values of photosynthetic productivity.

Insofar as photosynthetic tate is concerned, the author quite correctly complains of the insufficiency of investigations of this indicator, particularly during the morning hours. But along with this, A. A. Nichiporovich occasionally uses in his, in general, excellent work some rather poor examples. Thus, in illustrating the daily occurence of photosynthesis he mentions a number of times a single-peaked curve of photosynthesis. But it has already been established for twenty-five years by the investigations of E. F. Votchal that the daily course of photosynthesis has a many-peaked character. The old observations used by the author can be explained only by the fact that too large intervals were used between individual determinations of photosynthesis. With a decrease of intervals down to 15 minutes, the multiple peak of the photosynthetic curve is shown quite clearly. This nature of the curve is confirmed by the data of Bossian and others, in which photosynthesis was determined at 5-minute intervals. The theory of this phenomenon in many cases was developed by E. F. Votchal and was recently confirmed not only by the investigations of Bossian, but also by the data of the laboratory of the Ukrainian Institute of Plant Physiology.

Altogether, the question of the role played by the photosynthetic rate in amount of yield deserves wider discussion than was given in this work. In particular, the author did not touch on the question of the causes of the depression and stabilization of the daily course of photosynthesis, concerning the depression of photosynthesis in the field, and the necessity of studying the daily course of photosynthesis in the field.

Finally, the author comes to the question of the theoretically possible values of rate and productivity of photosynthesis as factors of yield. Analyzing the dark phase of photosynthesis and taking into account its rate, as in some cases, of 100 mg CO_2/ dm^2/ hour (instead of the average value of about 15-20 mg CO_2/ dm^2/ hour), the author does not find theoretical limits for the carrying capacity of the enzymatic phase. The author considers the important factor in realization of this capacity to be an increase in the utilization of the assimilants formed, which may be attained by developing a new variety with changed correlated interrelationships—a greater development of storage and reproductive organs. The analysis of the light phase of photosynthesis has also shown that there are still great possibilities for increase in the rate and productivity of photosynthesis. The calculations have shown that, instead of the 1-2% of solar radiation which is utilized by plants at the present time, it is possible to attain an increase in utilization up to 7-8%. The graph of energy utilization shows that the rate of photosynthesis may be raised to 250 mg (of course, only for individual, well-illuminated leaves) instead of 25 mg CO_2/ dm^2/ hour.

Constructing graphs on the basis of the considerations given, and using not even the uppermost limits of photosynthetic productivity, the author calculated the possibility of a daily increase in dry matter up to 1200 kg per hectare per day, and in biological yield up to 80 tons of dry matter for the growing period, which in relation to the sugar beet, for example, would have been expressed in a yield of edible roots to the amount of 2000 to 2400 centers per hectare.

In solving, in an original way, the problem of carbon dioxide nutrition for obtaining high yields, by means of exchanges of air layers through convection currents, the speed of which reaches 1 m/ minute, the author unexpectedly speaks abouth the possibility of lowering the CO_2 content by 10 to 20% in the leaf zone, which is hardly permissible on the basis of the calculations. Indeed, on the basis of experiments of the All-Union Sugar Beet Laboratory, with a wind velocity of 1 m/ second, the lowering of CO_2 content in the leaf zone at noon may reach 27%. This is also indicated by the data of Chapman, Gleasen, and Loomis cited by the author. Thus, in this question there seems to be some sort of discrepancy in the individual views of the author, especially as later, when speaking of the necessity of retaining the CO_2 evolved by the soil, the author writes that the plantings should be sufficiently dense for retention, but for ensuring currents, should not be too dense. In addition, one must note that the author for some reason persists in using foreign papers, ignoring some of our own investigation, qualitatively just as good.

Passing to the question of mineral nutrition, the author insists upon combining high doses of fertilizer, ensuring the saturation of soil by nutrient substances, with additional nutrition by easily assimilated fertilizer in the period which corresponds to the greatest requirements of plants. However, certain conditions are necessary for introducing these nutrient substances into the plant's metabolism; these conditions may be created, as was correctly noted, with the aid of rapid photosynthesis, which is the basic prerequisite of the best assimilation and the most effective utilization of high doses of fertilizers. In connection with this, the development of special methods of affecting photosynthesis and the development of new varieties with increased photosynthetic capacity is necessary. Thus, the most effective utilization of moisture will take place.

Together with the author, I see the significance of the work reviewed to be the fact that not only does it point out concrete methods of his investigation for further work in increasing yields, but also elucidates the practical possibility of a considerable increase in yield at the present time. It is necessary to emphasize, as a particular accomplishment of this book, an exceptional clarity in posing questions, and also the original treatment of a number of the situations mentioned.

A. S. Okanenko

NEWS OF SCIENCE

CONCERNING THE WORK OF PLANT PHYSIOLOGISTS IN KAZAKHSTAN

The rapid growth of agriculture in our Republic, linked with the successful reclamation of virgin soil and wastelands, as well as a need for the development and improvement of the forage basis for the growing cattle production, placed a number of new problems before the science of biology. In 1956, Kazakhstan became the second granary of the Soviet Union. We rejoice in this success, but at the same time it brings with it many obligations.

The central problem of plant physiology in our country at the present time is the development of the scientific basis of plant nutrition with the purpose of the greatest satisfaction of the plant's requirements in order to obtain high and stable yields (Academician A. L. Kursanov). The plant physiologists of the Republic, together with the biochemists, are trying to furnish actual practical aid to the Republic's agriculture by a systematic study of the life processes of plants. Along with this, the plan of scientific research also includes theoretical investigation, which would permit a profound evaluation of the state of a plant, the determination of its requirements, and the establishment of the significance of the phenomenon investigated in the life of the organism as a whole, and its interrelationship with its environment.

While planning scientific research in the Republic, in correlation with the decisions of coordinated meetings at the K. A. Timiriazev Institute of Plant Physiology, Academy of Sciences USSR, the realization of practical and theoretical problems encompasses the main division of plant physiology. In these one can include: the mineral nutrition and the activity of the root system of the plant; the mechanism of changes in water relations and their relationship to the other physiological processes of a growing and developing organism; the rate and efficiency of photosynthesis in relation to the genotypic characteristics of the plant and to the conditions of its cultivation; the respiration of plants in connection with their growth and development and also in connection with changes in the external environment; the mechanisms of ontogenetic changes of physiological and biochemical processes and processes reflecting progressive differentiation of the developing plant organisms; the integration of the plant, expressed by the processes of translocation of plastic substances and their reutilization in various stages of ontogenesis of the plant as a whole and of its specialized organs; the resistance of plants to unfavorable external conditions (drought, frost, etc) in relation to the characteristics of the plants and to methods of cultivation.

At the present time the group of physiologists and biochemists in the Institute of Botany, Academy of Sciences KazSSR is studying questions of practical significance, connected with the development of cereal agriculture, mostly in the Northern zone of newly reclaimed lands, with the increase of yield in corn and other feed plants in dry soil patches and irrigated agriculture, as well as with the increase of a number of non-forage crops.

Physiological and biochemical investigation of cereal crops in the basic areas of the Republic includes the following problems:

1) Physiology of drought resistance of hard and soft wheat in connection with pre-planting hardening against drought and high temperature (changes of colloid-chemical properties, the indicators of water relations and drought resistance and also of carbon and nitrogen exchanges in various phases of plant development).

2) Biochemical and technological characteristics of the grain in the basic varieties of winter and summer hard wheats, as well as of barley, in connection with the conditions of cultivation (carbon-amylase and protein-protease complexes of flour).

3) The effect of methods of improvement of solonetz soils * in the Northern zone of newly-reclaimed

*A dark, strongly alkaline soil.

lands, on the physiological functions of soft and hard wheat (indicators of water relations, efficiency of photosynthesis, constitution of soil elements, and indicators of carbon metabolism).

4) Characteristics of nitrogen nutrition of rice under conditions of constant flooding (carbon and nitrogen metabolism in connection with photosynthetic efficiency on the background of different conditions of nitrogen nutrition).

Investigations concerning the increase in productivity of corn and other feed crops are being carried out in the following directions: 1) the increase of drought resistance and yield production in the steppe zone of newly-reclaimed lands (Akmolinskaia Region) during cultivation of corn for silage (the effect of preplanting treatment and also of mineral nutrition elements on water relations of plants, on efficiency of photosynthesis, and on the indicators of carbon and nitrogen metabolism; establishment of the connection between photosynthetic efficiency and the thermal regime, as well as different areas of plant nutrition); 2) the interrelationship of water relations, rate of photosynthesis and metabolism in hybrid corn to the maximal yield under conditions of irrigated agriculture (investigation of rate and interlinking of physiological and biochemical processes during the basic steps of corn development under various water and nutritional regimes; ontogenetic changes of gradients of these processes and reutilization of plastic substances; the physiology of high-yield plants as a basis for further rationalization of the best agro-technique and raising of the yield of their photosynthetic activity); 3) the comparative study of seed crops (corn, Sudan grass, small grains, etc.) with various levels of water supply and preplanting treatment (physiology of drought resistance of these crops and methods of increasing their productivity in the zone of insufficient moisture).

The growth and development of, and the formation of useful substances in non-forage plants is investigated in the direction of: 1) a comparative physiological-biochemical investigation of variously-maturing varieties of cotton (characteristics of organ-forming processes, productivity of photosynthesis, and also carbon and nitrogen metabolism in various phases of plant development); 2) the formation of starch in potato tubers in relation to conditions of cultivation (change of productivity of photosynthesis, activity of phosphorylase and phosphoglucomutase, amylolytic activity, as well as starch content in tubers in various stages of development); 3) a physiological investigation of and increase of frost resistance in Eucommia in connection with its acclimatization in Kazakhstan (changes of the growth rhythm in relation to age of plants, as well as separate indicators of metabolism, reflecting the degree of preparedness of plants for winter conditions); 4) the content of alkaloids and other substances in some species of Ephedra (comparative investigation of the widely-distributed species of Ephedra in relation to the accumulation of ephedrine in different phases of plant development and the search for a connection between the accumulation of alkaloids and that of tannic substances); 5) the physiological gradient as an indicator of integration, progressive differentiation and metabolism of the developing tobacco plant (study of the leaf in relation to physiological-biochemical indicators in connection with the phases of development, different regimes of mineral nutrition and surgical treatment).

The Department of Plant Physiology of Kazakh Governmental University carries out an investigation of: various methods directed toward increase in yield and improvement of grain quality; the effect of methods of millet planting on carbon and nitrogen metabolism; the establishment of critical soil moisture for the most important varieties of millet cultivated in the northern regions of the Republic; the effect of carbonates, applied in supplementary feeding, on water relations, photosynthesis, yield and quality of corn grain; the effect of antibiotics and fungicides on wheat blight.

The Department of Botany of the Agricultural Institute investigates the following problems: water relations and productivity of photosynthesis in various varieties of corn in the dry-steppe zone of newly-reclaimed lands; water relations and drought resistance of various woody species under various soil-climatic conditions; seasonal changes of physiological processes and characteristics of several stone-fruit varieties, in relation to their winter resistance in the Alma-Ata fruit zone.

Finally, the Department of Botany of the Alma- Ata Pedagogical Institute is investigating the effect of microelements (boron, zinc, manganese, and others) on the productivity of sugar beet, corn, vegetable crops and forage grasses; it is also making an investigation of the ecological-phylogenetic characteristics of the spectral brightness of terrestrial plants in connection with the problem of plants on Mars (radiation regime of various species of plants in an ecological profile from the Zailiiskie mountains of Ala-tau to the deserts of the Southern Pribalkhash'e, which is studied by the method of comparative spectrophotometry).

Such is the direction of the work which is being carried out at the present time by the plant physiologists of the Republic's institutions.

The development of physiological investigations in coming years should be based on the organization of the physiological laboratories in the system of the Kazakh affiliate of VASKhNIL, which will permit the physiologists of the Academy of Sciences KazSSR to relax work on the physiology of particular plants, at the same time establishing more profound investigation of the general and theoretical problems of plant physiology. The planned organization of the Institute of Plant Physiology and Biochemistry in the system of the Academy of Sciences KazSSR should contribute to such a direction for future work.

L. G. Dobrunov

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

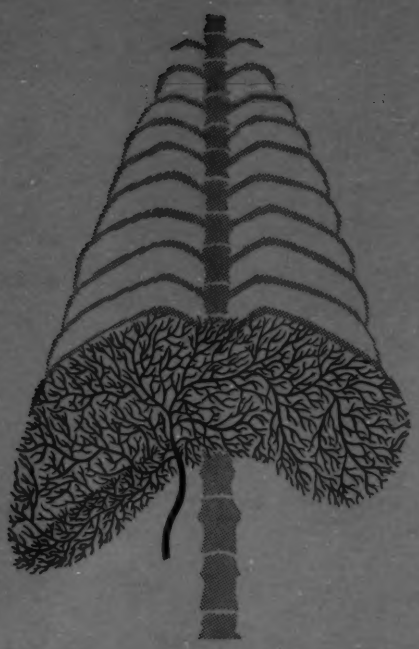
Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology



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Origins of Angiospermous Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. 68 pgs. Ready now. $3.00 (U.S. & Canada) $3.50 (Foreign)

Essays on the Evolutionary Morphology of Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. Ready November 1958. $5.00 (U.S. & Canada) $5.50 (Foreign)

Problems in the Classification of Antagonists of Actinomycetes. By G. F. Gause. Edited by David Gottlieb. Translated by Fritz Danga. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

X-Rays and Plants. By L. P. Breslavets. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

Arachnida. Vol. IV, No. 2. Fauna of the U.S.S.R. By B. I. Pomerantzev. Edited by George Anastos. Translated by Alena Elbl. Ready early 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

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